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Vol. 104, Jan. - 1981
119 Subblement

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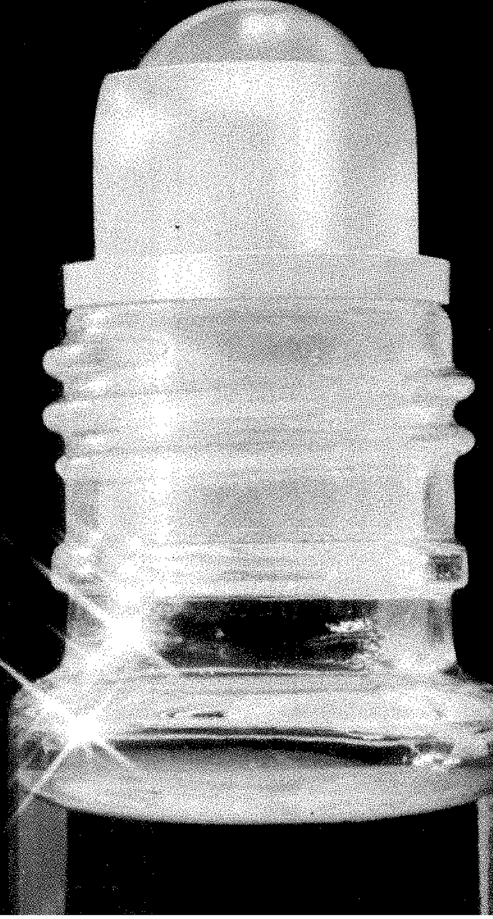

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Percutaneous Absorption of Steroids

edited by P. Mauvais-Jarvis, C.F.H. Vickers and J. Wepierre
September 1980, xviii + 294pp., £15.60 (UK only) / $36.00, 0.12.480680.5

International specialists in dermatology, steroid biochemistry, endocrinology and gynaecology discuss the theoretical basis and the practical applications of percutaneous absorption of steroid hormones in human beings.

Behçet's Syndrome

Clinical and Immunological Features
edited by T. Lehner and C.G. Barnes
1979, xii + 324pp., £16.40 (UK only) / $38.00, 0.12.442680.8

The aim of this book, which records the proceedings of a conference sponsored by the Royal Society of Medicine in London, is to assist the large number of specialists who may encounter one or more of the manifestations of Behçet's syndrome to make a correct diagnosis. The contributors describe in detail the various clinical manifestations, discuss the diverse therapeutic approaches and deal at length with recent advances in the immunological investigation of Behçet's syndrome.

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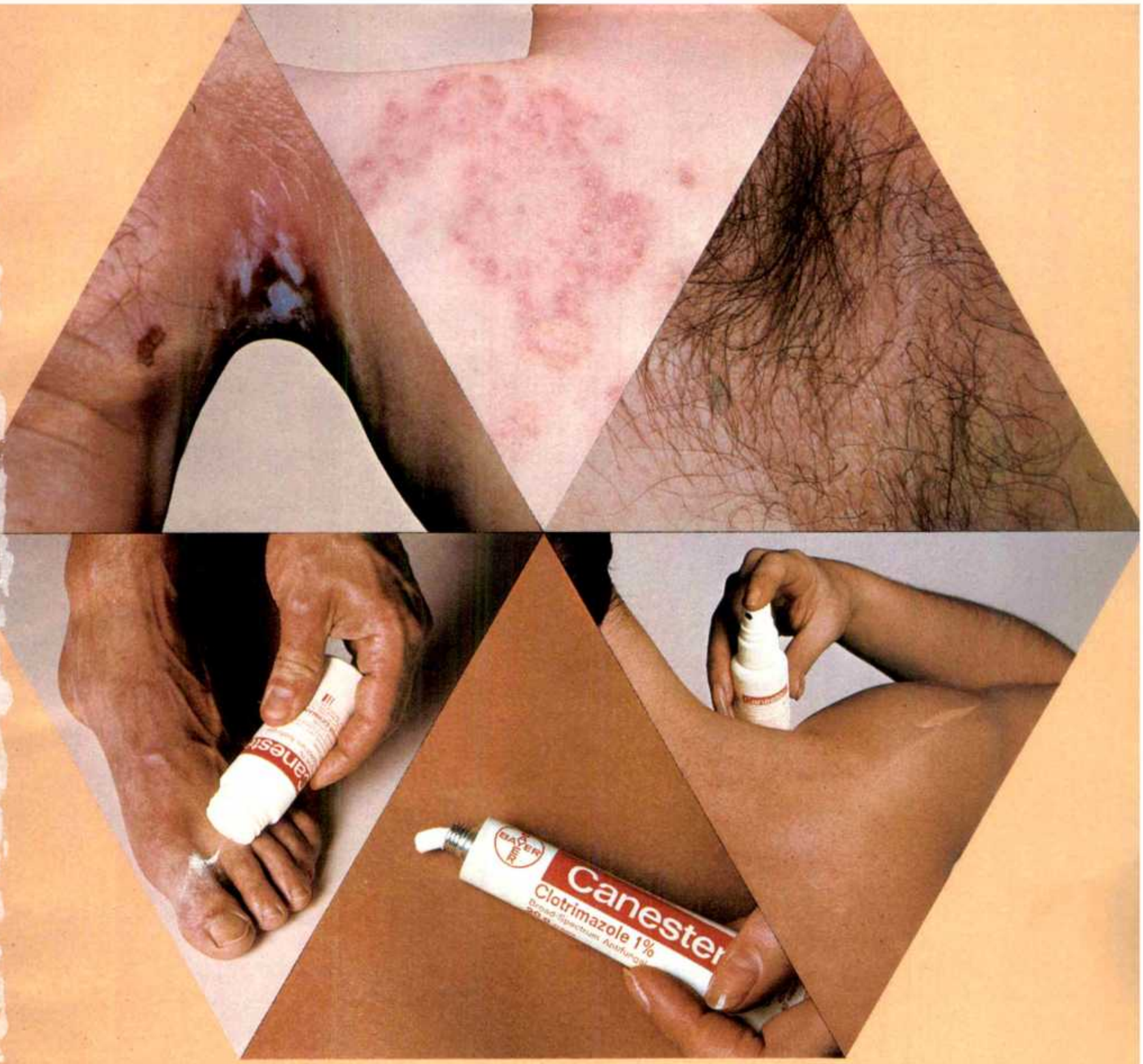

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ser Spray: PL 0010/0060 Powder: PL 0010/0067 **Basic NHS Prices** Cream: 20g, £1.52 50g, £3.55 Atomiser Spray: 40ml, £4.08 Powder: 30g, £1.10. Further information
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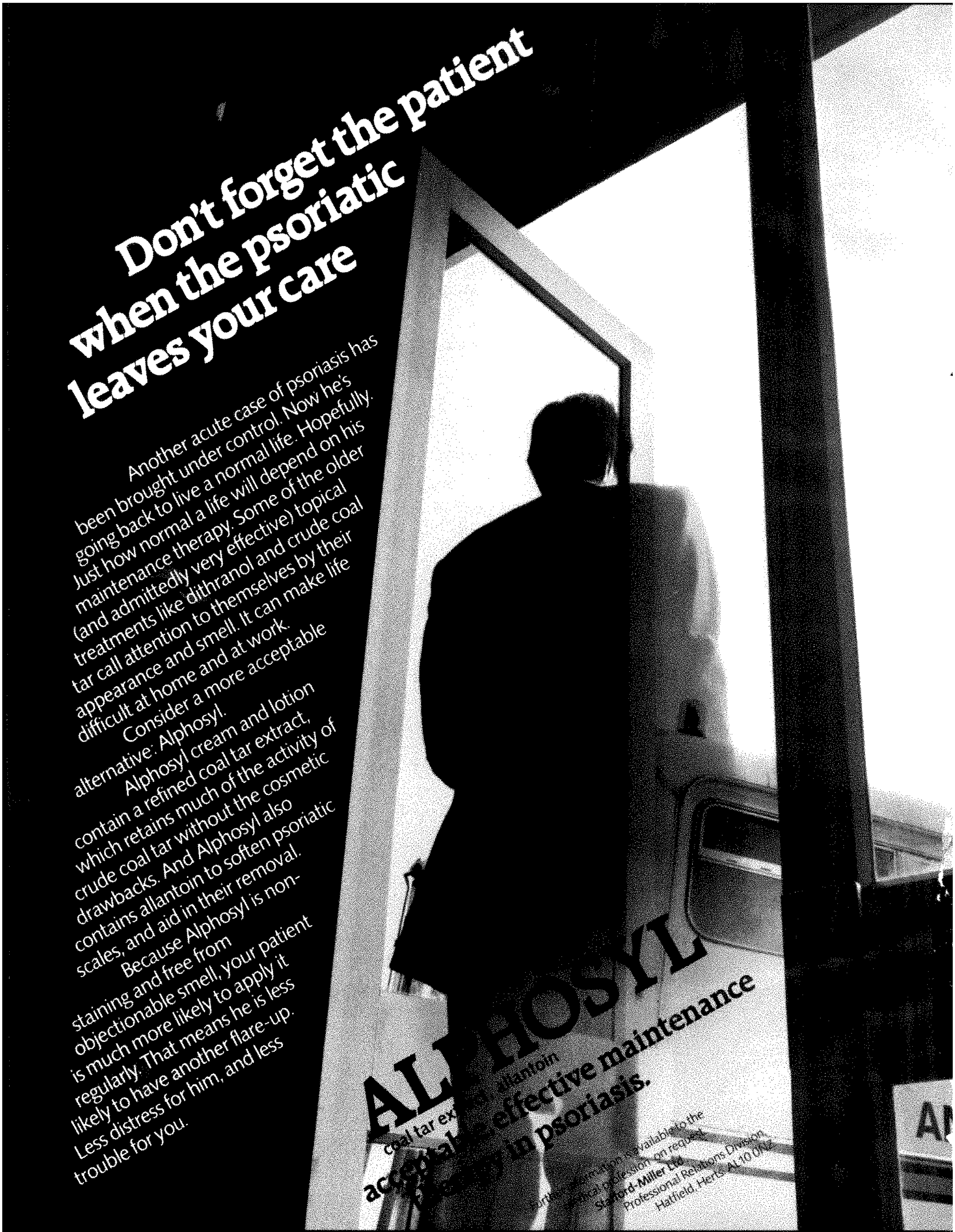

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Presentation LOTION AND CREAM Containing allantoin 2%, refined alcoholic extract of coal tar 5%, in a greaseless vanishing-cream base. The lotion is a light, free-flowing emulsion and the cream is smooth and homogeneous, light tan in colour. **Uses** Dermatological stimulant for the treatment of psoriasis. **Dosage and Administration** Apply liberally two to four times daily, massage well into the affected areas. Occlusive dressings are unnecessary. **Contra-indications and Warnings, etc.** For external use only. Discontinue if irritation occurs or in cases of sensitivity to coal tar. **Pharmaceutical Precautions** Do not refrigerate. **Legal Category** No legal restrictions on sale or supply. **Package Quantities** ALPHOSYL LOTION: Bottles of 250ml. ALPHOSYL CREAM: Tube of **Further Information** Alphosyl Lotion is the basic treatment for psoriasis of the body and scalp. Alphosyl Cream is designed especially for lubrication in the intertrigenous areas. **Product Licence**

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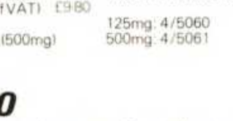

THE INSIDE STORY ON FUNGAL INFECTION

Prescribing information

Dosage and administration

Doses should be taken after meals for maximum absorption.

Adults: normally 500 to 1,000mg daily in divided doses, but not less than 10mg/kg bodyweight daily.

Children: usually 10mg/kg (5mg/lb) bodyweight daily in divided doses.

Duration of treatment: for hair or skin at least four weeks' treatment is required, whereas finger or toe nails may need six to twelve months' treatment. Continue for at least two weeks after all signs of infection have disappeared.

Contra-indications

Porphyria or severe liver disease.

Griseofulvin may cause deterioration in some patients with liver disease and liver function should be monitored in such conditions.

Precautions

Griseofulvin may decrease the effect of coumarin anticoagulants. Absorption is inhibited when phenobarbitone is taken concurrently. The blood level, and hence efficacy, may also be impaired as the result of concurrent administration of substances such as phenylbutazone and sedative and hypnotic drugs which induce metabolising enzymes. In pregnant animals, administration of very high doses of griseofulvin can cause foetal abnormalities. The relevance of this finding to humans has not been established, but use during pregnancy is best avoided.

Side effects

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Further information

Grisovin is not effective in infections caused by *Candida albicans* (monilia), Aspergilli, *Malassezia furfur (Pityriasis versicolor)* and Nocardia species.

Basic NHS cost	Product Licence numbers
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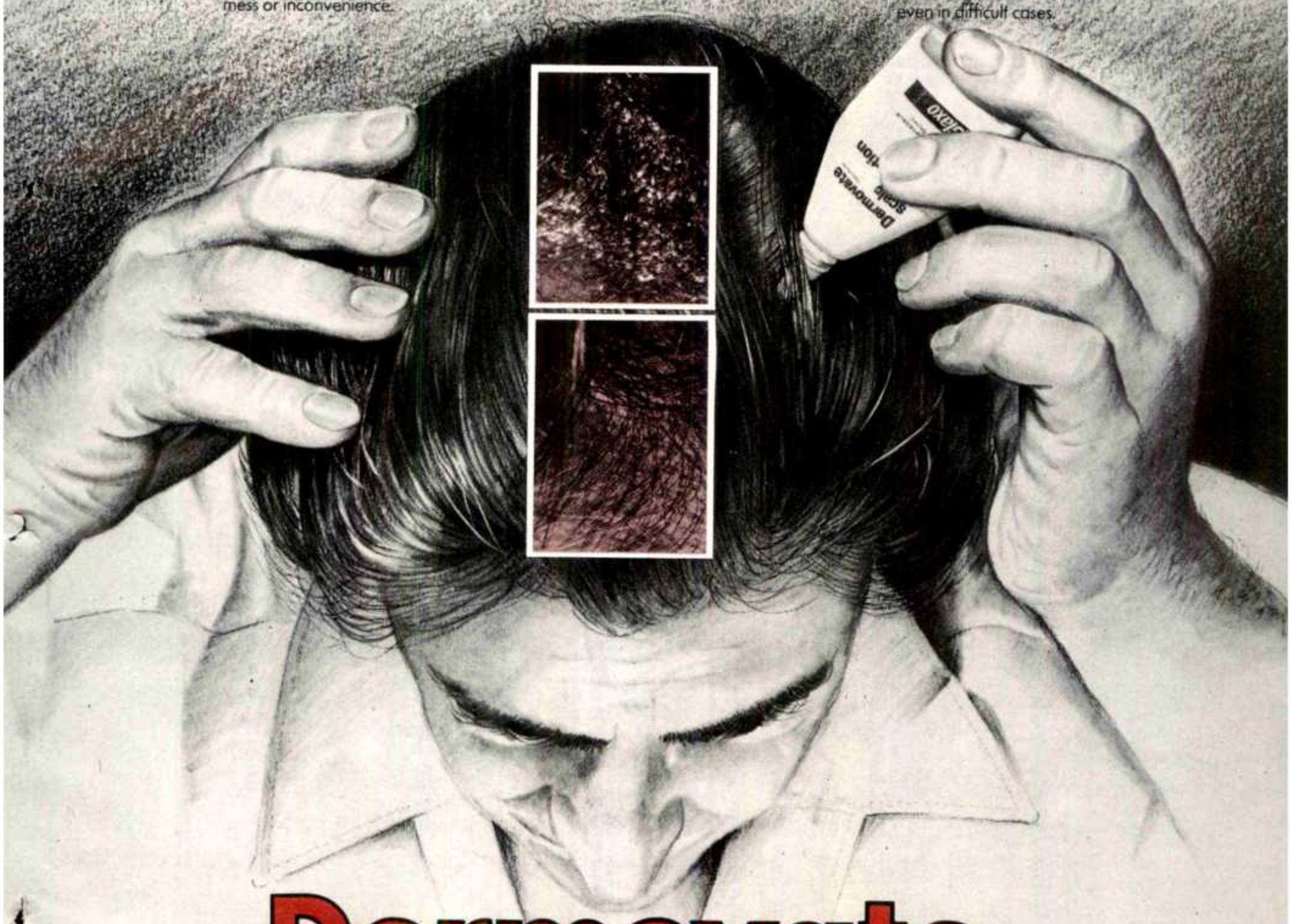

Dermovate

(clobetasol propionate)

Scalp Application

Prescribing information

Uses

Psoriasis and recalcitrant eczemas of the scalp. Clobetasol propionate is a highly-active topical corticosteroid which is indicated for use in short courses for conditions which do not respond satisfactorily to less active steroids.

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Apply sparingly to the scalp night and morning until improvement occurs. As with other highly-active topical steroid preparations, therapy should be discontinued when control is achieved. Repeated short courses of Dermovate Scalp Application may be used to control exacerbations. If continuous steroid treatment is necessary, a less potent preparation should be used.

Contra-indications

Infections of the scalp. Hypersensitivity to the preparation.

Precautions

Care must be taken to keep the preparation away from the eyes. Do not use near a naked flame. Long-term continuous therapy with Dermovate Scalp Application should be avoided, particularly in infants and children, in whom adrenal suppression occurs readily.

Development of secondary infection requires withdrawal of topical corticosteroid therapy and institution of appropriate systemic antimicrobial therapy.

Topical administration of corticosteroids to pregnant animals can cause abnormalities of fetal development.

In general, topical steroids should not be used extensively in pregnancy, i.e., in large amounts or for prolonged periods.

Side effects

Dermovate preparations are usually well tolerated, but if signs of hypersensitivity appear, application should be stopped immediately. As with other topical corticosteroids, local atrophy may result from prolonged treatment, particularly if occlusive dressings are used. When extensive areas are treated, sufficient systemic absorption may occur to produce the features of hypercorticism, especially in infants and children. This effect is more likely to occur after prolonged treatment, particularly if occlusive dressings are used. In rare instances, treatment of psoriasis with corticosteroids (or its withdrawal) is thought to have provoked the pustular form of the disease.

Presentation

Plastic squeeze bottle with elongated nozzle containing 25 or 100ml

Basic NHS cost (exclusive of VAT)

25ml bottle £2·72
100ml bottle £9·65

Product Licence number

4/0242

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Administration: following the dressing chart, apply a protective ointment to the skin area bordering the ulcer to protect normal skin. Place dressings saturated with Benoxyl 20 Lotion in intimate contact with ulcer surfaces.

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Pack and NHS price: bottles of 100ml, £3.00

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Arachis Oil extract of crude coal tar	0.3%
Oleyl alcohol	1.00%

Polytar Plus contains 3.0% hydrolysed animal protein, in addition to the above.

Uses Polytar Liquid and Polytar Plus are indicated in the treatment of scalp disorders such as psoriasis, seborrhoea, dandruff, eczema and pruritus. They are also of value in the removal of pastes and pomades used in the treatment of psoriasis.

Dosage and Administration The hair should be wetted and sufficient applied to produce an abundant lather. The scalp and adjacent areas should be vigorously massaged with the fingertips. The hair should then be thoroughly rinsed and the procedure repeated.
Polytar Liquid or Polytar Plus should be used once or twice weekly.

Contra-indications, warnings, etc. Nil

Pharmaceutical Precautions Store in a cool place

Packs and Prices

	Pack	Basic NHS price
Polytar Liquid	65ml	57p
	150ml	82p
	350ml	£1.48
	1 litre	£3.90
Polytar Plus	150ml	90p

Product Licence Number
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Full information and Data sheet on request.

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Dithrocream

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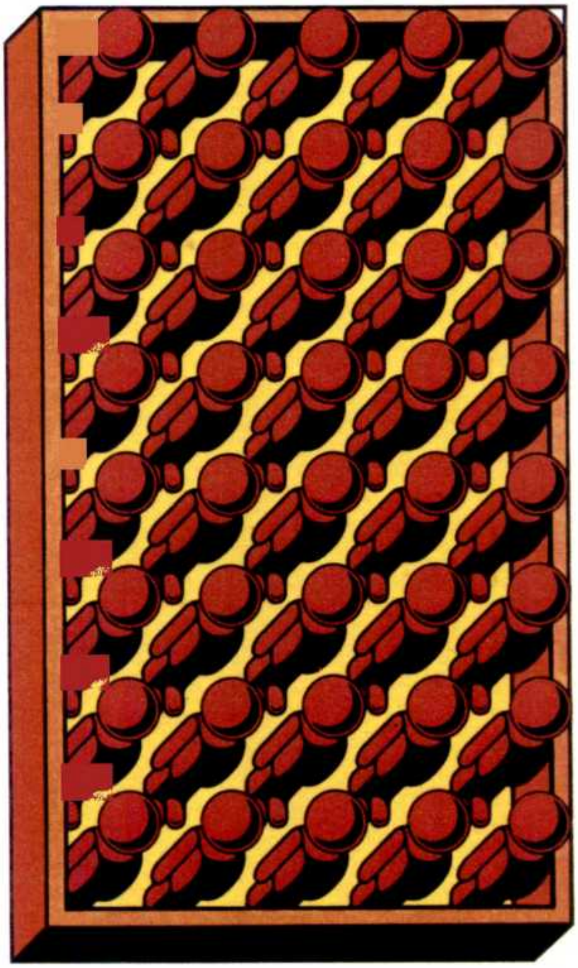
Natural selection!



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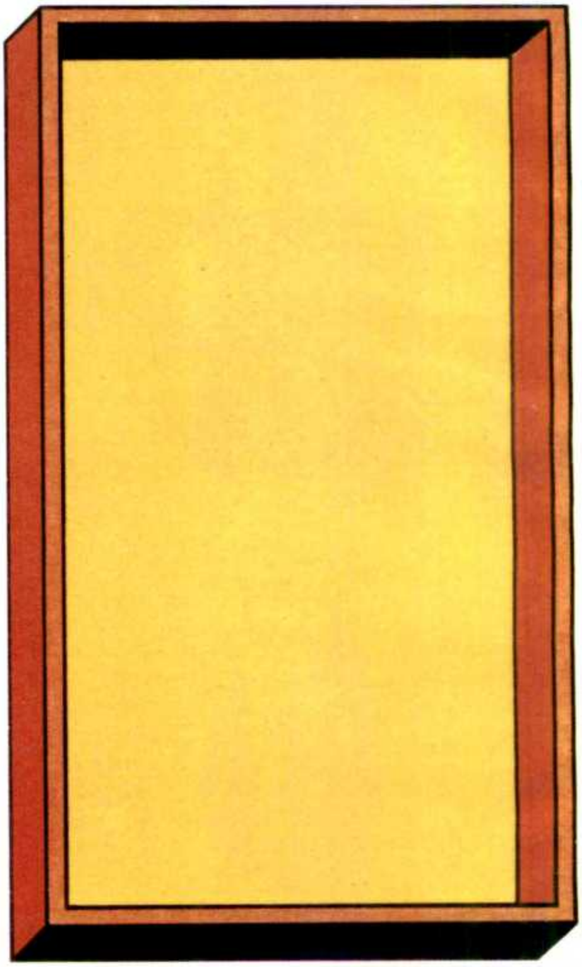

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Dithrocream Forte: pale yellow aqueous cream containing 0.5% Dithranol B.P.

Indications
For the topical treatment of subacute and chronic psoriasis including psoriasis of the scalp. Dithrocream Forte should only be used for resistant cases.

Directions for use
Apply sparingly to the psoriatic lesions preferably at night and rub in gently and carefully until absorbed. Bathe before the next application. For scalp lesions, shampoo before use. Continue treatment until the skin is smooth and clear.

Contraindications
Acute psoriasis; dithranol sensitivity.

Precautions
Keep away from the eyes. Apply to psoriatic areas only. Use cautiously after recent application of potent steroids. Wash the hands after use.

Basic N.H.S. prices:
Dithrocream 0.1% 50gm tubes £1.81
Dithrocream 0.25% 50gm tubes £1.96
Dithrocream Forte 50gm tubes £2.19

Product Licence Nos.

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Dithrocream 0.25%	PL 0173/0028
Dithrocream Forte (0.5%)	PL 0173/0027

References:
1. *British Journal of Dermatology*, 1979, **100**, 475

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British Journal of Dermatology (1981) **104**, 1.

Clinical and Laboratory investigations

A method for culturing human hair follicle cells

P.J.J.M.WETERINGS*, A.J.M.VERMORKEN* AND H.BLOEMENDAL†

* Research Unit for Cellular Differentiation and Transformation, and † Department of Biochemistry, University of Nijmegen, Nijmegen, The Netherlands

Accepted for publication 19 May 1980

SUMMARY

For the first time a method for culturing human hair follicle cells is described. The bovine eye lens capsule, a basement membrane-like structure, is used as the substrate for the cultures. In a culture medium supplemented with hydrocortisone and insulin about 70% of the original follicles will form growing colonies of diploid keratinocytes.

Human hair follicles are currently in use as a biopsy material for the detection of some genetic diseases (Vermorken *et al.*, 1980). In these so-called inborn errors of metabolism the disease is caused by a decreased activity of one of the cellular enzymes that can be determined in isolated hair follicles. Certain experimental approaches, however, such as studies on potential mutagenicity of carcinogenic agents, demand the use of culture systems. Fibroblasts have generally been used for this purpose, but since about 90% of all malignancies are carcinomas, i.e. of epithelial origin, the need for culture models of epithelial cells is acute (Reid & Rojkind, 1979). The human hair follicle represents an ideal biopsy tissue for these studies, not only since it originates from the epithelium but also because it can easily be removed without any permanent damage to the tissue or appreciable pain for the donor. When we started our experiments, human hair follicle cells had never been brought into culture. We now have developed a method for culturing these cells. Moreover we show that the cells are diploid and therefore useful for genetic studies.

MATERIALS AND METHODS

Preparation of the culture dish

Lenses were isolated from calf eyes obtained fresh from the slaughterhouse. The anterior part of the capsule of the lens was removed carefully using a pair of scissors. These capsules were incubated for 15 min at 37°C in 0·3% trypsin in a balanced salt solution lacking calcium and magnesium. After thorough rinsing in balanced salt solution supplemented with penicillin and streptomycin (100 μg/ml

Correspondence: Dr A.J.M.Vermorken, University of Nijmegen, Geert Grooteplein Noord 21, 6500 HB Nijmegen, The Netherlands.

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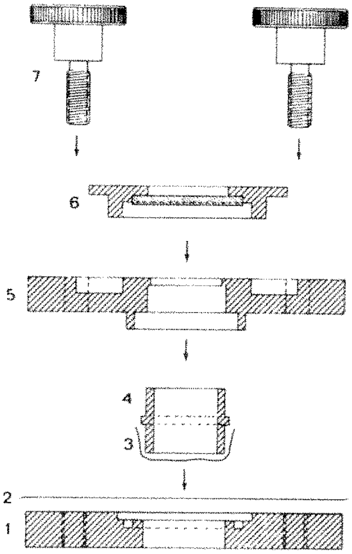

FIGURE 1. Schematic drawing of the culture dish, showing how the various parts are put together. Essentially, the bottom of the dish (1) is covered by a square piece of foil (2). Then the central cylinder (4) on which the lens capsule (3) is stuck, is placed on the bottom. The cylinder is forced through the opening of the bottom by gentle pressing of the upper part of the dish (5). After placing the lid (6) the various parts are fixed together by means of four screws (7). The glass centre of the lid allows microscopic examination of the cultures under sterile conditions.

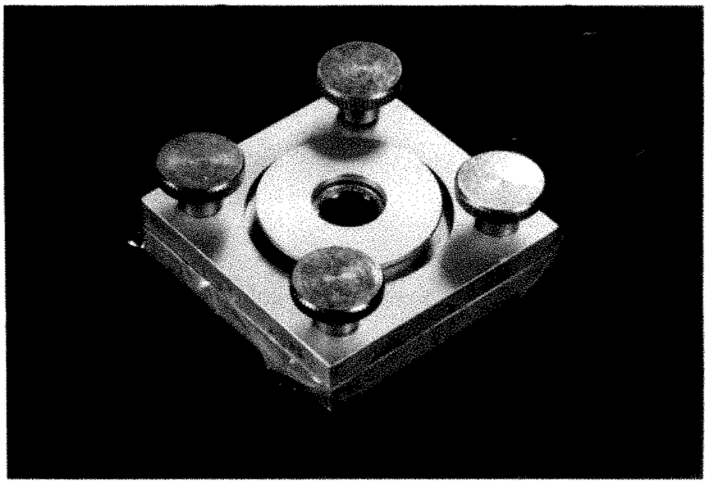

FIGURE 2. Picture of the mounted culture dish.

each) the capsules were irradiated with X-rays at a dose of 10,000 rad using a cobalt source. These capsules were placed in culture dishes as developed by Hukkelhoven, Vermorken & Bloemendal (1980). These culture vessels allow the use of the bovine eye lens capsule, a material resembling the basement membrane of the skin, as a growth substrate. Figure 1 shows the way in which the various parts of the dish are put together. The mounted dish is shown in Fig. 2.

Tissue preparation and cultivation

Hair follicles were plucked from several areas of the scalp and only those in the anagen phase with intact bulb and sheath were used. The follicles were immediately immersed in a balanced salt solution supplemented with penicillin and streptomycin (100 μg/ml each). Hair follicles were placed on the capsules and cultivation was performed using Minimal Essential Medium (Eagle) with 25 mM Hepes and supplemented with 15% fetal calf serum (Sera Lab, Sussex, England), 0.4 μg/ml hydrocortisone and 4 μg/ml bovine insulin (a gift from Dr Pieterson, Organon International B.V.).

Cell processing for flow cytometry

After 3 weeks of growth the original follicles were removed from the culture vessels. The remaining colonies were treated with a solution of 0.3% trypsin (Difco 1:250) and 1% EDTA for 15 min at 37°C. A suspension of single cells was then obtained by rigid pipetting. This suspension was centrifuged for 5 min at 900 **g**. The cell pellet was prepared for flow cytometry and analysed according to the method of Bauer *et al.* (1980).

RESULTS

After 2 to 4 days of culturing, cells apparently originating from the outer root sheath of the hair follicle start growing on the capsules. In Fig. 3 a colony is shown after 2 weeks in culture. The cell

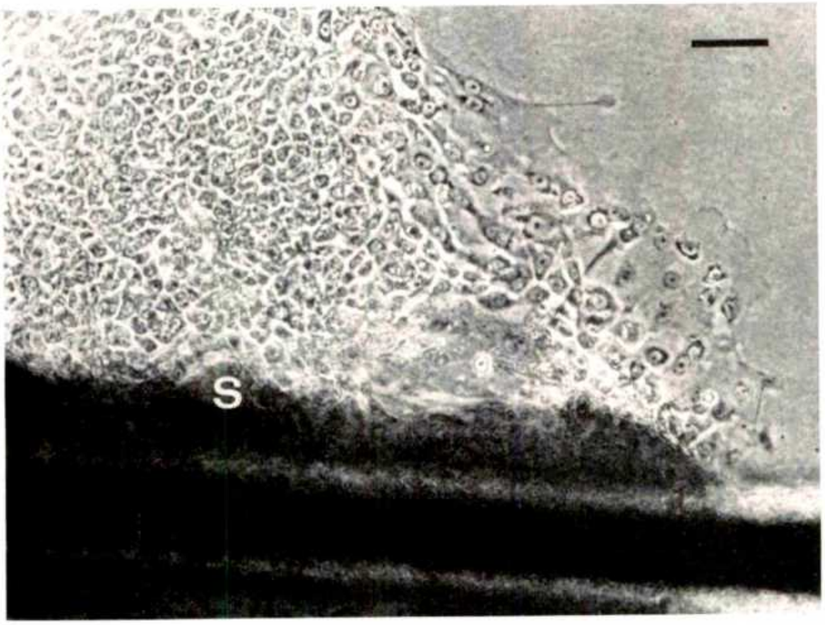

FIGURE 3. Hair follicle cells after 2 weeks in culture (bar: 100 μm). S = Outer root sheath. The boundary of the colony is one cell layer in thickness. The photograph was taken using a Leitz-diavert phase-contrast microscope.

TABLE 1. Percentage of hairs forming keratinocyte colonies after 4 days in culture

	Hairs on plastic	Hairs on lens capsule
Minimal Essential Medium + 15% serum	—	+/—
Minimal Essential Medium + 15% serum + hydrocortisone	+/—	+/++
Minimal Essential Medium + 15% serum + insulin	+/—	+
Minimal Essential Medium + 15% serum + hydrocortisone + insulin	+/—	++

—	0–10%
+/—	10–30%
+	30–50%
+/++	50–70%
++	70–100%

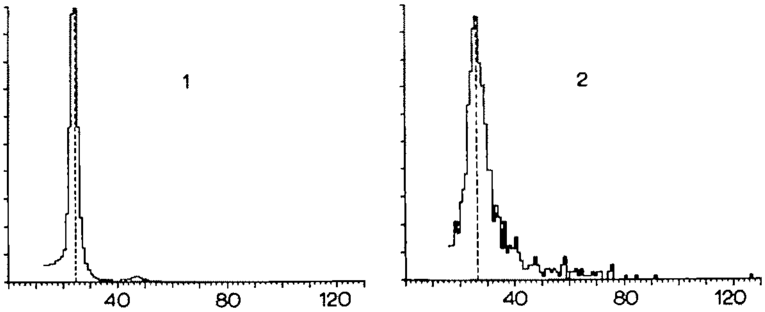

FIGURE 4. Histograms obtained upon flow cytometry of cells originating from: 1—freshly plucked hair follicles; 2—cultures after 3 weeks of growth. Abscissa: channel number.

boundaries are indistinct since most of the culture is now stratified and several layers in thickness. About 70% of the follicles placed on the lens capsules will give rise to a colony of growing keratinocytes. The bovine eye lens capsule, a biological substrate resembling the basement membrane, plays an essential role in determining this rate of success. On the plastic bottom of culture dishes very small colonies are formed only incidentally. Addition of insulin and hydrocortisone is also essential for obtaining a high rate of growth (see Table 1).

The results of the flow cytometry experiments are shown in Fig. 4. It is evident that the cultured cells are diploid.

DISCUSSION

Cultured hair follicle cells may offer new possibilities for the early detection of some inherited diseases (Vermorken *et al.*, 1980). When we started our experiments it was generally accepted that under cell culture conditions keratinocytes depend on the support of fibroblasts for colony formation (Kondo, Aso & Nauta, 1979; Rheinwald & Green, 1975a, b; Rheinwald & Green, 1977). Our finding that hair follicle cells can be cultured without the support of living fibroblasts has interesting implications for genetic studies. Not only is this culture method of epithelial cells highly relevant for some cancer studies, but also the fact that colonies can be obtained from individual hair follicles, that in most cases have been shown to originate from one single cell (Gartler *et al.*, 1969), offers for the first time an easy method to obtain cell cultures of clonal origin.

NOTE ADDED IN PROOF

While this paper was in preparation we also demonstrated that the protein patterns of the cultured hair follicle cells and the intact hair follicles are identical (Weterings, Vermorken & Bloemendal, 1980).

ACKNOWLEDGMENTS

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REFERENCES

BAUER, F.W., CROMBAG, N.H.C.M.N., DE GROOD, R.M. & DE JONGH, G.J. (1980) Flow cytometry as a tool for the study of cell kinetics in epidermis. *British Journal of Dermatology*, **102**, 629.

GARTLER, S.M., GANDINI, E. ANGIONI, G. & ARGOLAS, N. (1969) Glucose-6-phosphate dehydrogenase mosaicism: utilization as a tracer in the study of the development of hair root cells. *Annals of Human Genetics*, **33**, 171.

HUKKELHOVEN, M.W.A.C., VERMORKEN, A.J.M. & BLOEMENDAL, H. (1980) A novel method for culturing epithelial cells on a biological substrate. *Preparative Biochemistry*, in press.

KONDO, S., ASO, K. & NAUTA, M. (1979) Culture of normal human epidermal cells with 3T3 feeders on millipore filters. *Journal of Investigative Dermatology*, **72**, 85.

REID, L.M. & ROJKIND, M. (1979) New techniques for culturing differentiated cells: reconstituted basement membrane rafts. In: *Methods in Enzymology, Vol. LVIII* (Ed. by S.P.Colowick and N.O.Kaplan), pp. 263–278. Academic Press, New York.

RHEINWALD, J.G. & GREEN, H (1975a) Formation of a keratinizing epithelium in culture by a cloned cell line derived from a teratoma. *Cell*, **6**, 317.

RHEINWALD, J.G. & GREEN, H. (1975b) Serial cultivation of strains of human epidermal keratinocytes: the formation of keratinizing colonies from single cells. *Cell*, **6**, 331.

RHEINWALD, J.G. & GREEN, H. (1977) Epidermal growth factor and the multiplication of cultured human epidermal keratinocytes. *Nature*, **265**, 421.

VERMORKEN, A.J.M., WETERINGS, P.J.J.M., DE BRUYN, C.H.M.M. & GEERTS, S.J. (1980) Human hair follicles in biomedical research. In: *Current Problems in Dermatological Research, Vol.* 9 (Ed. by J.W.H.Mali). Karger, Basel.

WETERINGS, P.J.J.M., VERMORKEN, A.J.M. & BLOEMENDAL, H. (1980) Protein biosynthesis in cultured human hair follicle cells. *Molecular Biology Reports*, in press.

British Journal of Dermatology (1981) **104**, 7.

The histopathology and ultrastructure of liver disease in erythropoietic protoporphyria

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SUMMARY

The light, polarization and electron microscopic changes in the liver of four cases of erythropoietic protoporphyria (EPP) are described. In one of these cases liver involvement resulted in death, whereas in the other three there were no symptoms referable to hepatic disease. In two cases hepatic pigment was readily visible on light and polarization microscopy and corresponded to characteristic crystalline aggregates seen ultrastructurally. Pronounced micronodular cirrhosis and cholestasis were present in the fatal case, while in the other only mild periportal fibrosis and mononuclear inflammatory cell infiltrate occurred. The third and fourth cases showed no evidence of hepatic involvement on light microscopy. Electron microscopy, however, revealed characteristic crystal-containing vacuoles indicative of protoporphyrin deposition. The need for early detection of the hepatic lesion is emphasized.

Since the original characterization of EPP (Magnus *et al.*, 1961) it has become apparent that the clinical features of extreme skin photosensitivity may be complicated by liver disease (Redeker, Bronow & Sterling, 1963; Langhof *et al.*, 1964; Cripps & Scheuer, 1965; Kniffen & Porter, 1971; Mascaro *et al.*, 1971; Klatskin & Bloomer, 1974; Matilla & Molland, 1974; Wolff, Wolff-Schreiner & Gschnait, 1975; Cripps & Goldfarb, 1978) which is sometimes fatal (Barnes, Hurworth & Millar, 1968; Donaldson *et al.*, 1971; Schmidt & Stich, 1971; Iwanow *et al.*, 1972; Thompson *et al.*, 1973; Scott *et al.*, 1973; Meffert *et al.*, 1974; Bloomer *et al.*, 1975; Meyer *et al.*, 1975; MacDonald & Nicholson, 1976; Cripps, Gilbert & Goldfarb, 1977; Cripps & Goldfarb, 1978).

The importance of serious liver disease in EPP has been slow to gain prominence and the belief persists that cirrhosis or liver damage of any type occurs only in the exceptional case. This impression may well be false and is undoubtedly due to the frequent absence of clinical evidence of hepatic disease until the pathology has reached an advanced stage (Barnes *et al.*, 1968; Donaldson *et al.*, 1971; MacDonald & Nicholson, 1976). Over 25% of affected cases show some histological change of hepatic fibrosis in the absence of clinical evidence of liver disease while 65% show light microscopical evidence of hepatic protoporphyrin deposition (Cripps & Goldfarb, 1978).

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CASE HISTORIES

Patient 1 (MacDonald & Nicholson, 1976) (male, aged 53 years) had suffered from childhood from swelling and blistering of the skin within 20 min of exposure to sunlight, resulting in coarsening and pitted scarring of the skin of face and dorsa of hands. The condition was diagnosed as EPP in 1961. At that time liver function tests were normal, faecal protoporphyrin was 1210 μg/g of dry stool, coproporphyrin excretion was 127 μg/g dry stool and red cell porphyrins were 3000 μg/100 ml packed cells. Monochromator testing (Magnus *et al.*, 1959) showed maximum reactivity at 400 nm.

Skin biopsy showed gross deposition of homogenous periodic acid Schiff (PAS) positive material around superficial dilated blood vessels.

Investigation of the family revealed that a twin sister and daughter were affected.

Until 1974 the erythrocyte protoporphyrin ranged from 2000 to 4000 μg/100 ml packed cells; the faecal protoporphyrin was constantly elevated sometimes with moderately raised coproporphyrin, but liver function tests results remained normal.

Early in 1974 the patient became unwell with a history of increased photosensitivity, malaise, lassitude, abdominal pain, dark urine and pale stools. Examination revealed mild icterus and gross hepatosplenomegaly. At this time serum bilirubin level was 34 mg/100ml, while aspartate aminotransferase (AST), lactate dehydrogenase (LDH) and alkaline phosphatase (alk. phos.) levels were elevated at 180 iu/l, 225 iu/l and 104 iu/l respectively. The only other abnormal findings were prolongation of the prothrombin time and gross oesophageal varices visible on films of a barium swallow. Clinical and biochemical deterioration was rapid thereafter; red cell protoporphyrin reached a maximum of 6020 μg/100 ml cells, bilirubin level reached 85 mg/100ml and levels of AST, LDH and alk. phos. respectively reached 475, 302 and 108 iu/l. Analysis of faecal and urinary porphyrins at this time showed the presence of uroporphyrin, hexa-, hepta-, penta-carboxyl porphyrins, coproporphyrin and suburoporphyrins. Isocoproporphyrins were not detected in either stool or urine.

Photosensitivity intensified with increase in protoporphyrin levels. Hepatic encephalopathy developed and the patient died suddenly after massive haemorrhage from oesophageal varices.

Patient 2 (male, aged 20 years) since birth had experienced photosensitivity, particularly through glass, with symptoms of pruritus, burning, erythema and painful swelling shortly after sun exposure. An infiltrated yellowish appearance of the cheeks and nose with small scars gradually appeared throughout childhood and adolescence while the skin of the dorsa of the hands became wrinkled and hyperkeratotic. The clinical features of EPP were obvious on initial examination in 1977.

Skin biopsy from the dorsum of the hand showed mixed hyper- and parakeratosis. A wide band of homogenous material which stained positively with PAS surrounded dilated capillaries of the mid- and superficial dermis. This material fluoresced positively in direct immunofluorescence studies. Investigation of blood count and levels of serum and urinary iron, serum calcium, phosphorus, copper, caeruloplasmin, cholesterol, triglycerides, urea and electrolytes, cryoglobulin and immunoglobulins were normal. Of the liver function tests, serum bilirubin, prothrombin level and serum proteins were normal. The serum glutamate-oxalate transminase (SGOT), serum glutamate-pyruvate transminase (SGPT) and alk. phos. levels were elevated, however, at 74 iu/l, 91 iu/l and 390 iu/l respectively; lactate dehydrogenase, α-hydroxybutyrate dehydrogenase and creatine phosphokinase levels were normal. The bromsulphthalein retention test was abnormal. α-Fetoprotein level was markedly elevated at 65·3 ng/ml. No biliary calculi could be demonstrated on cholescystography. Laparoscopy showed the liver to be enlarged, smooth, firm and green-black in colour.

Porphyrin studies showed, in erythrocytes, maximum levels of 1510 μg/100 ml packed cells of protoporphyrin and, in the faeces, 1585 μg/g dry stool of protoporphyrin, 1058 μg/g dry stool of coproporphyrin fraction and 192 μg/g dry stool of porphyrin X.

Patient 3 (female, aged 14) had experienced cutaneous symptoms from the age of 4 months when she began screaming within 5 min of being in the sun.

Since that time she has suffered from burning and swelling of the skin after sun exposure. Blisters frequently developed on the nose, cheeks, dorsa of the hands and back of the knees. Lesions healed with pitted scarring. Symptoms improved somewhat after puberty and were also improved with treatment of β-carotene canthaxanthin, 200 mg per day.

Skin biopsy showed characteristic cuffing of dermal blood vessels by PAS positive material which fluoresced postively with fluorescein conjugated anti-immunoglobulin antisera. Erythrocytes showed typical protoporphyrin red autofluorescence. Haematological and biochemical investigations including routine liver function tests and bromsulphthalein retention test were normal.

Patient 4 (female, aged 22) from the age of 18 months has suffered from swelling and erythema of the skin associated with a burning sensation within 10 min of sun exposure. Symptoms, which persist for several days, are relieved by application of cold water. The condition was diagnosed as EPP in 1973. Over the years small pitted scars have developed on the face associated with a yellowish waxy appearance. Linear scars are also present over the knuckles. Symptoms have been considerably improved by β-carotene canthaxanthin 120 mg-200 mg/day.

Skin histology revealed a homogenous perivascular band of PAS-positive material characteristic of porphyria. The majority of erythrocytes showed porphyrin fluorescence consistent with EPP. Liver function tests including bromsulphthalein retention test were normal and other investigations were unremarkable.

LIVER HISTOLOGY

Materials and methods

A percutaneous liver biopsy was obtained from patient 1 during the terminal illness using a Vim-Silverman needle and post mortem liver tissue was also available. The specimens were fixed in 10% formal saline and embedded in paraffin. Sections were stained with haematoxylin and eosin (H & E), PAS, Gordon and Sweet's silver stain for reticulin, Perl's stain for haemosiderin, by the Fouchet method for bile and Schmorl's stain for lipofuscin. Unstained sections were examined under ultraviolet light from a 74 watt Xenon light source to detect porphyrin fluorescence. The sections were examined by light and polarization microscopy. Samples of post-mortem material were fixed in buffered glutaraldehyde-formaldehyde-sucrose, post-fixed in osmium tetroxide and embedded in TAAB resin. Ultrathin sections were cut on an LKB 3 ultramicrotome, stained with uranyl acetate and lead citrate and examined in a Corinth 275 electron microscope. From patients 2 and 3, percutaneous liver biopsy specimens were similarly prepared for light, fluorescence and polarization microscopy. Tissue from patient 2 was fixed in 1·5% glutaraldehyde, post-fixed in 1% osmium tetroxide, embedded in Epoxy resin, sectioned on a Reichert OMU 3 ultramicrotome, stained with uranyl acetate and lead citrate and examined in Hitachi HU 12 A and Philips EM 300 electron microscopes. Tissue from patients 3 and 4 were fixed in 2% glutaraldehyde, post-fixed in 1% osmium tetroxide, embedded in TAAB resin, sectioned on an LKB 3 ultramicrotome, stained with uranyl acetate and lead citrate and examined in a Hitachi HU 12 A electron microscope.

Results

In patient 1, the liver was macroscopically enlarged with an atrophic left lobe and hypertrophic right lobe, was black-brown in colour and exhibited obvious cirrhosis (Fig. 1).

Microscopically, particularly obvious in H & E (Fig. 2) and reticulin stained material, there was micronodular cirrhosis with complete disruption of the normal liver architecture and moderate bile

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duct proliferation. Individual hepatocytes frequently appeared multinucleate or possessed large abnormal hyperchromatic nuclei while the cytoplasm showed features of feathery degeneration (Fig. 3). Collections of mononuclear cells were randomly distributed among the fibrous tissue where it formed septa between 'regeneration lobules'. Specimens stained by H & E showed deposition of rust brown material as fine granules or clumps within hepatocytes and coarser deposits within Kupffer cells (Fig. 3). Plugs of pigment were also present in dilated bile canaliculi and ducts and in hepatic sinusoids. The amount of pigment varied from area to area and between individual hepatocytes. Staining for bile by the Fouchet method revealed that some of the pigment plugs in canaliculi and bile ducts gave a positive green reaction, while others stained brown or a brown-green colour (Fig. 4) suggesting that there was cholestasis in addition to stasis of some other pigment. Perl's reaction for haemosiderin was negative, the PAS stain was unremarkable, but deposits of lipofuscin in some hepatocytes were revealed by Schmorl's stain.

Polarization microscopy of H & E stained sections revealed dramatic birefringence of the pigment material. Large deposits exhibited a 'Maltese Cross' appearance while small granular deposits appeared as brilliant reflections conferring a 'starry sky' aspect (Fig. 5). Fluorescence microscopy of unstained liver showed transitory red protoporphyrin fluorescence (Fig. 6).

Ultrastructural study showed the striking presence of collections of crystals within hepatocytes, Kupffer cells and free in canaliculi. The crystals in hepatocytes lay in dense sheaves or feathery patterns within non membrane-bound vacuoles containing relatively electron lucid material resembling lipid (Fig. 7a). In many cells similar lipid-like vacuoles devoid of crystals were also present, in addition to irregular masses of lipofuscin. Hepatocyte mitochondria were numerous with well-developed cristae. No protoporphyrin crystals were detectable in intact mitochondria but occasional vacuoles containing few crystals showed membranous remnants similar to mitochondrial cristae.

Many biliary canaliculi and ductules were plugged by crystalline aggregates similar to those in hepatocytes (Fig. 7b) and phagocytosed protoporphyrin deposits were seen in some Kupffer cells. Collagen bundles around small groups of cells were prominent and occasionally incorporated trapped crystals.

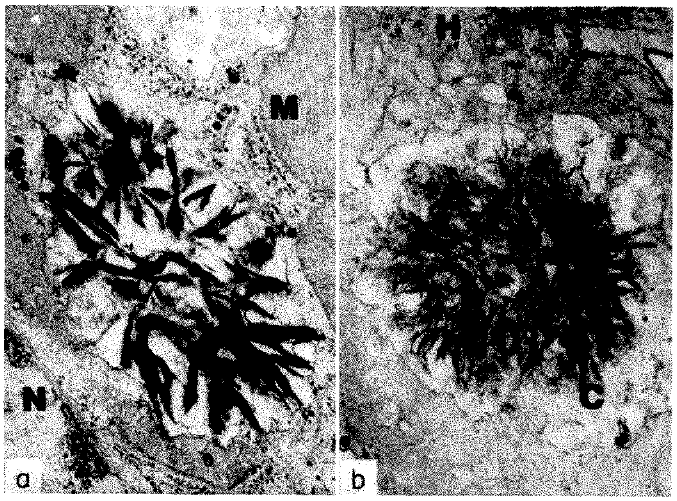

FIGURE 7. (a) Patient 1: aggregates of needle-like crystals of protoporphyrin within vacuole in hepatocyte: M—mitochondrion; N—nucleus (E.M. × 40,000). (b) Patient 1: dense collection of protoporphyrin crystals within canaliculus: C—canaliculus; H—hepatocytes (E.M. × 40,000).

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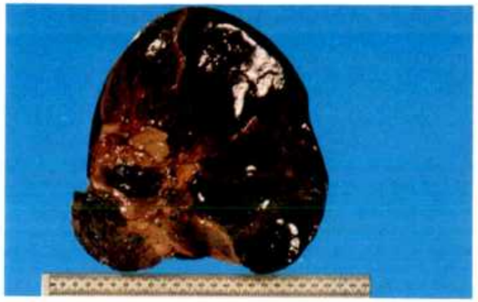

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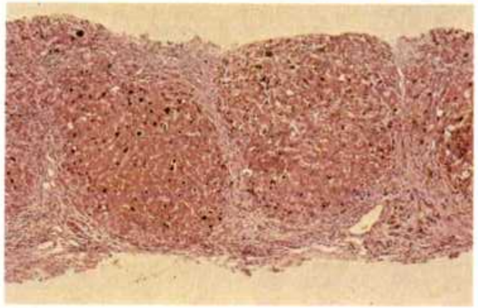

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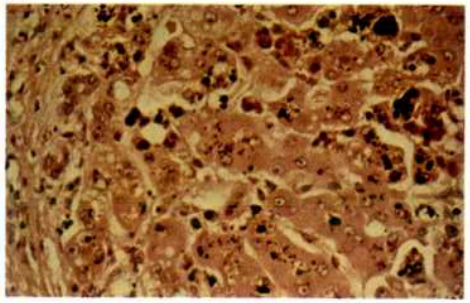

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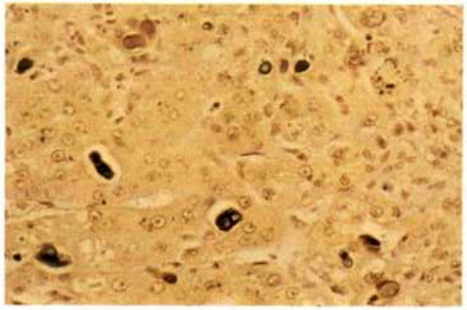

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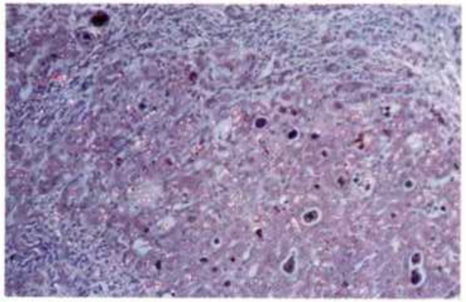

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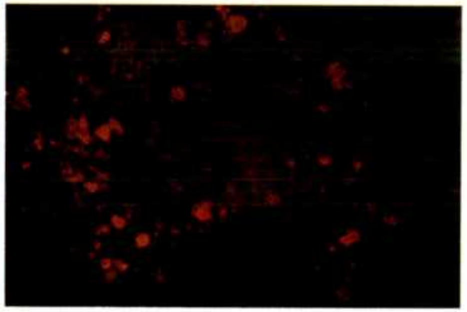

FIGURE 1. Patient 1: liver at autopsy. The liver, brown-black in colour, exhibits obvious cirrhosis with an atrophic left lobe and hypertrophic right lobe.

FIGURE 2. Patient 1: ante-mortem needle liver biopsy. Wide bands of fibrous tissue and inflammatory cell infiltrate separate hepatic regeneration lobules. Pigment deposition is visible in ductules and hepatocytes (H & E × 15).

FIGURE 3. Patient 1: pigment deposition in hepatocytes and canaliculi. Many hepatocytes are multinucleate and show features of 'feathery degeneration' (H & E × 85).

FIGURE 4. Patient 1: stasis of two types of pigment—protoporphyrin (brown) and bile (green)—within dilated canaliculi (Fouchet Stain × 85).

FIGURE 5. Patient 1: characteristic 'Maltese cross' and 'starry sky' birefringence of protoporphyrin on polarization microscopy (H & E × 40).

FIGURE 6. Patient 1: characteristic red protoporphyrin autofluorescence in the liver (unstained × 70).

Massive deposits of protoporphyrin (819 μg/g) were found on biochemical analysis of post mortem liver material. Coproporphyrin, uroporphyrin, hepta-, hexa-, penta- and suburo-porphyrin as well as small amounts of 'X' peptidoporphyrin were also present.

In patient 2, the liver architecture was essentially normal although there was some widening of portal tracts by a mononuclear cell infiltrate and mild periportal fibrosis. The presence of brown pigment was readily visible in hepatocytes, canaliculi and Kupffer cells, either as clumps or fine granules.

Fouchet and Perl's reactions were negative and no lipofucsin was demonstrable.

Polarization microscopy revealed an appearance similar to that in patient 1. Fluorescence microscopy of deparaffinised sections revealed characteristic red fluorescence of protoporphyrin.

Ultrastructurally, signs of cytolysis were evident in some hepatocytes while others appeared normal. The altered cells showed increase and dilatation of the endoplasmic reticulum, particularly around the nucleus, irregularly placed swollen mitochondria and numerous cytoplasmic vacuoles whose size and number increased according to the degree of cytolysis (Fig. 8). Myelin figures were occasionally seen. Cells showing greater degrees of damage seemed to be those situated in the region of the portal tracts.

Some hepatocytes contained vacuoles of a different nature whose size varied from 1–4 μ and which were usually not membrane-limited, although some were bounded by a dense zone around most of the vacuole (Fig. 9). These vacuoles contained relatively electron-lucid material arranged in sheets, pools or peripheral rings which suggested the appearance of lipid (Fig. 9). More electron-dense material in masses or filaments was situated randomly within the vacuoles (Fig. 9). At very high resolution (× 150,000–250,000) the dense material was seen to comprise needle-shaped crystals which showed a tendency to layer together in groups of two to five parallel elements forming alternating dark and light bands (Fig. 10). The average diameter of the dark bands was 25 Å. In the region

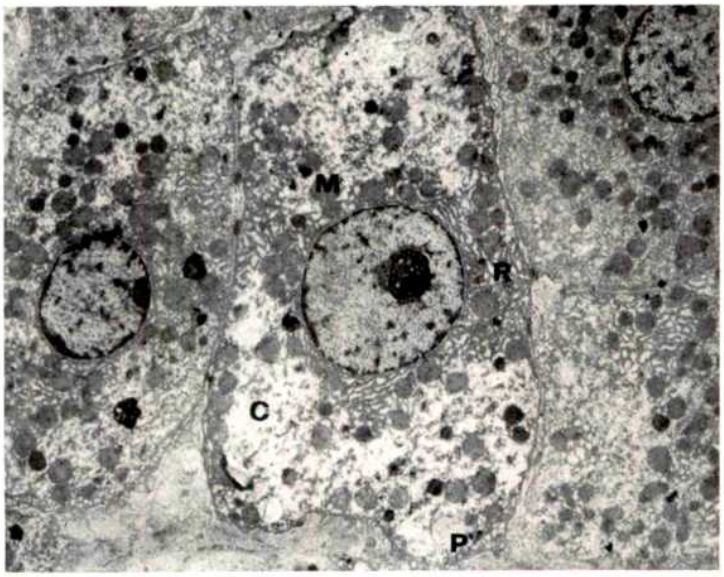

FIGURE 8. Patient 2: hepatocytes showing cytolytic changes—cytoplasmic vacuoles (C), dilated endoplasmic reticulum (R), irregularly placed swollen mitochondria (M) and crystal-containing vacuoles (P) (E.M. × 4,000).

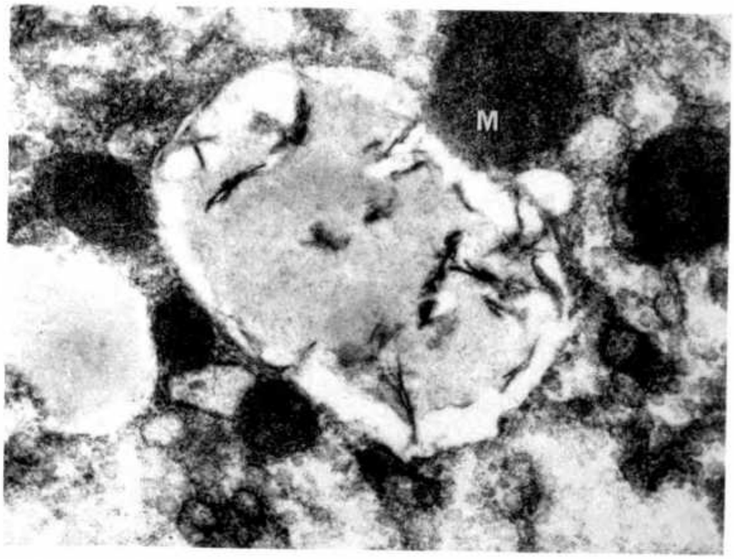

FIGURE 9. Patient 2: vacuole containing electron dense granules and crystals of protoporphyrin lying in a pool of lipid-like substance. Swollen structureless mitochondria (M) connect with the vacuole (E.M. × 33,000).

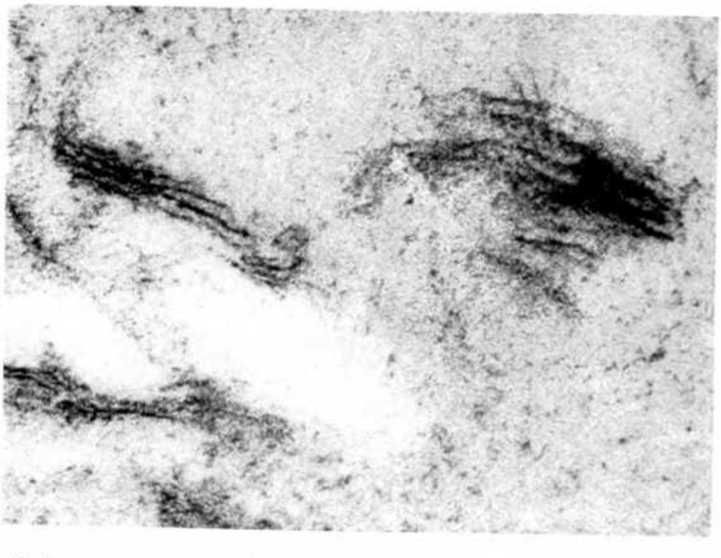

FIGURE 10. Patient 2: granules and layered needle-shaped crystals of protoporphyrin within vacuole (E.M. × 250,000).

of the crystals numerous fine dense granules of diameter 2–25 Å were present (Fig. 10). Many showed a tendency to linear arrangement forming apparently the early crystalline elements (Fig. 10).

Mitochondria were frequently grouped around the crystal vacuoles and occasionally were seen in direct contact without interposition of any membrane (Fig. 11a). Some of the vacuoles themselves showed structures which may represent remnants of cristae of mitochondria (Fig. 11b).

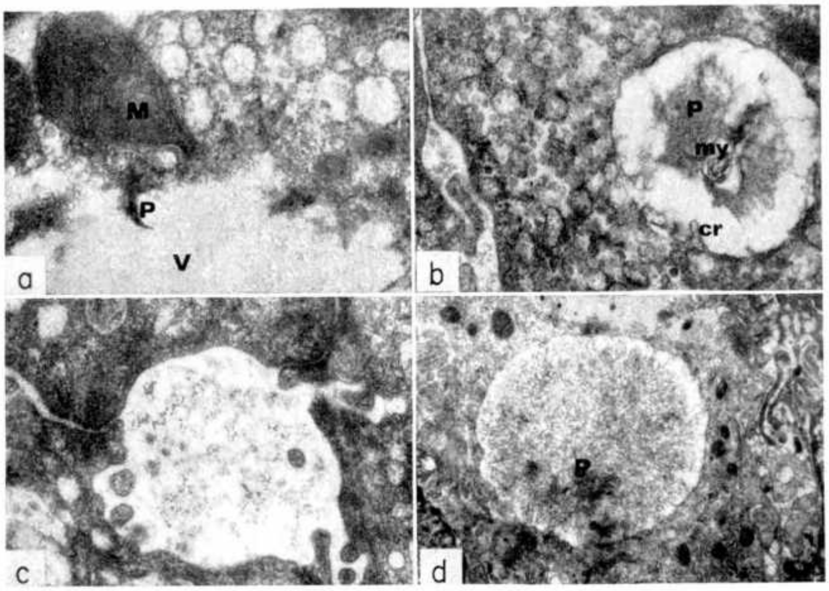

FIGURE 11. (a) Patient 2: mitochondrion (M) connecting directly with vacuole (V): P—protoporphyrin crystal (E.M. × 30,000). (b) Patient 2: protoporphyrin containing vacuole with membranous remnants suggestive of mitochondrial cristae (cr): my—myelin figure; P—Protoporphyrin (E.M. × 30,000). (c) Patient 2: granular protoporphyrin within canaliculus (E.M. × 28,000). (d) Patient 2: phagocytosed protoporphyrin (P) within Kupffer cell. (E.M. × 10,000).

Electron-dense granular material was also seen in sinusoids and canaliculi (Fig. 11c) and, in places, small crystalline forms seemed to arise from this. Occasionally large plugs of crystals were seen in ductules. A few Kupffer cells showed aggregates of crystals in autophagosomes (Fig. 11d) but were found rarely and only after prolonged observation.

Light microscopy of H & E stained sections of the liver of patient 3 failed to reveal any abnormality. No porphyrin autofluorescence was detectable under ultraviolet light and polarization microscopy was unremarkable.

Ultrastructurally, however, many hepatocytes in certain areas of the liver contained crystal-laden vacuoles similar to those seen in the first two cases. Several such vacuoles were sometimes seen within a single liver cell. The vacuoles were not membrane limited and contained electron-dense granules which were aggregated together to form needle-like crystalline structures and amorphous masses (Fig. 12a). Mitochondrial membranes closely adjacent to the crystal-containing vacuoles occasionally appeared to be ruptured (Fig. 12a). Other signs of hepatocyte cytolysis were not prominent. Crystals were not observed in canaliculi or Kupffer cells.

Light, fluorescence and polarization microscopy of the liver tissue of patient 4 also was unremarkable.

Ultrastructurally, some hepatocytes were seen to contain vacuoles which included crystalline aggregates of protoporphyrin similar to those previously described. Again several vacuoles were occasionally present within a single hepatocyte (Fig. 12b).

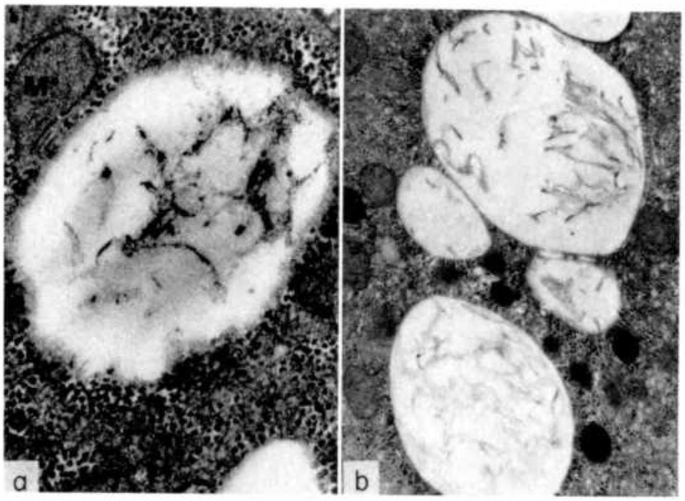

FIGURE 12. (a) Patient 3: crystals and masses of protoporphyrin in non-membrane bound vacuole. An adjacent mitochondrion (M) appears to discharge into the vacuole (E.M. × 60,000). (b) Patient 4: protoporphyrin-laden vacuoles within hepatocyte (E.M. × 28,000).

DISCUSSION

The most important early histopathological characteristic of protoporphyrin liver disease has been considered to be deposition within hepatocytes, canaliculi and Kupffer cells of brown granular material (Porter & Lowe, 1963; Cripps & Scheuer, 1965; Barnes *et al.*, 1968; Donaldson *et al.*, 1971; Scott *et al.*, 1973; Thompson *et al.*, 1973; Matilla & Molland, 1974; Wolff *et al.*, 1975; MacDonald & Nicholson, 1976; Bruguera *et al.*, 1976; Cripps & Goldfarb, 1978) which may be readily distinguished from bile, iron and lipofuscin. The material exhibits a characteristic birefringence on polarization microscopy and unstained specimens exhibit a red autofluorescence when viewed with ultraviolet light (Thompson *et al.*, 1973; Klatskin & Bloomer, 1974; Wolff *et al.*, 1975; Bruguera *et al.*, 1976; Cripps & Goldfarb, 1978). A gradient of severity of hepatic changes may be found on biopsy of individual cases. Post-mortem examination of several cases (Barnes *et al.*, 1968; Donaldson *et al.*, 1971; Bloomer *et al.*, 1975; Cripps *et al.*, 1977) revealed changes comparable to those in patient 1 including hepatocyte degeneration and necrosis, micronodular cirrhosis, bile duct proliferation, cholestasis and porphyrastasis in addition to the cellular pigment deposits already described. Routine tests of liver function and even the relatively sensitive bromsulphthalein retention test have often been normal until shortly before death in many of these cases (Donaldson *et al.*, 1971; Bloomer *et al.*, 1975; MacDonald & Nicholson, 1976). Protoporphyrin deposition with perhaps modest inflammatory infiltrate and fibrosis, as in patient 2 of this study, is likely to be the only visible abnormality on light microscopy of early liver involvement. The presence of protoporphyrin deposits in the liver has been demonstrated in more than 65% of the cases biopsied by Cripps & Goldfarb (1978).

Ultrastructural study of our cases also showed widely varying severity of pathological changes. In the mildly affected early cases, despite only mild degenerative changes in hepatocytes, intracellular vacuoles containing crystals of characteristic size and shape (Matilla & Molland, 1974; Klatskin &

Bloomer, 1974; Wolff *et al.*, 1975; Bruguera *et al.*, 1976) differing from those found in porphyria cutanea tarda (Waldo & Tobias, 1973) were readily seen. Whereas dense aggregated sheaves of crystals were found in hepatocytes, canaliculi and Kupffer cells in our fatal case, in the early cases crystals could be found only with difficulty in sinusoids, canaliculi or Kupffer cells although their presence within hepatocytes was obvious. The relative sparsity of obvious protoporphyrin crystals in canaliculi and Kupffer cells in patient 2 was surprising in view of the light microscopical appearances but suggests that the important diagnostic lesion ultrastructurally is the presence of crystals within hepatocytes, which undoubtedly appear in this site before their passage to sinusoids and canaliculi and their phagocytosis by Kupffer cells. Our patients 3 and 4 indicate that at least some and perhaps all of the 35% of EPP sufferers who do not exhibit protoporphyrin deposition on light microscopy—as indicated by the study of Cripps & Goldfarb (1978)—nevertheless show the hepatic lesion ultrastructurally.

Despite lack of definitive evidence, our study supports the view that crystals arise initially in mitochondria. We have observed membrane-like structures resembling cristae within vacuoles and also direct connection between vacuoles and obvious mitochondria. Further, vacuoles of lipid-like substance similar to those seen in EPP arise as a result of mitochondrial degeneration (Perrot & Ortonne, 1978).

In the griseofulvin-fed mouse model for protoporphyria the crystals appear earliest in the mitochondria of hepatocytes and have been shown to consist of protoporphyrin (Matilla & Molland 1974; Gschnait *et al.*, 1975). Undoubtedly the appearances seen ultrastructurally in our cases resemble progressive stages of protoporphyrin hepatic damage in the mouse model. The findings of liver cell damage with dense sheaves of crystals within hepatocytes and canaliculi are analogous to those seen between 2 and 5 weeks of griseofulvin feeding in mice. We were unable to find truly membrane-bound vacuoles and conclude that our early cases correspond either to a stage later than 5 weeks of griseofulvin in the mouse or that the crystal vacuoles in man differ from those of the mouse model. Wolff *et al.*, (1975) and Bruguera *et al.*, (1976) also found little, if any, evidence of a limiting membrane to the crystal vacuoles in man. Indeed comparison of the hepatic damage in EPP and the mouse model may be misleading as the latter also differs biochemically in showing elevated urinary delta aminolaevulinic acid, porphobilinogen and coproporphyrin. Porphyrinuria is absent in EPP except in late severe disease (Barnes *et al.*, 1968; Donaldson *et al.*, 1971; Schmidt & Stich, 1971; Scott *et al.*, 1973; Thompson *et al.*, 1973; MacDonald & Nicholson, 1976) and is probably attributable to the presence of cirrhosis (Doss *et al.*, 1972).

Accumulation of protoporphyrin may arise as a result of mitochondrial deficiency of ferrochelatase which is responsible for conversion of protoporphyrin to haem. This enzyme is known to be deficient in bone marrow, peripheral erythrocytes (Bottomley, Tanaka & Everett, 1975; de Goeij, Christianse & Van Steveninck, 1975) and leukocytes (Brodie *et al.*, 1977), liver and cultured skin fibroblasts (Bonkowsky *et al.*, 1975). The severity of liver disease may be related to the degree of ferrochelatase deficiency. Severity of cutaneous symptoms of EPP has been shown to vary, however, in cases with comparably deficient activity of the enzyme (Bloomer *et al.*, 1976). Recently, instability rather than deficiency of ferrochelatase has been postulated in EPP (Becker *et al.*, 1977) and various exogenous factors may affect this.

Currently there is no method of predicting serious liver disease in EPP. Further ultrastructural studies of the liver in EPP may show that protoporphyrin deposition is invariable. Only serial histological studies can relate the morphological changes to the ultimate prognosis. Although death from protoporphyrin liver disease has been reported at the ages of 11 (Cripps *et al.*, 1977) and 16 (Meyer *et al.*, 1975) it has most commonly occurred in middle-age following swiftly after initial detection of biochemical evidence of hepatic dysfunction.

The rapidity of onset of hepatic disease probably relates to the rate of accumulation, within hepatocytes, of crystals whose presence causes progressive liver damage (Gschnait *et al.*, 1975; MacDonald & Nicholson, 1976). As cholestyramine has been found to remove protoporphyrin from the liver (Kniffen, 1970; Davidson, Bloomer & Klatskin, 1973) it is important that clinicians be aware of the need to detect the early hepatic lesions by liver biopsy and that pathologists should be acquainted with the characteristic histopathological findings which may be reliably detected by light and electron microscopy.

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REFERENCES

BARNES, H.D., HURWORTH, E. & MILLAR, J.H.D. (1968) Erythropoietic porphyrin hepatitis. *Journal of Clinical Pathology*, **21**, 157.

BECKER, D.M., VILJOEN, J.D., KATZ, J. & KRAMER, S. (1977) Reduced ferrochelatase activity: a defect common to porphyria variegata and protoporphyria. *British Journal of Haematology*, **36**, 171.

BLOOMER, J.R. PHILLIPS, J.M., DAVIDSON, D.L. & KLATSKIN, G. (1975) Hepatic disease in erythropoietic protoporphyria. *American Journal of Medicine*, **58**, 869.

BLOOMER, J.R., BONKOWSKY, H.L., EBERT, P.S. & MAHONEY, M.J. (1976) Inheritance in protoporphyria. Comparison of haem synthetase activity in skin fibroblasts with clinical features. *Lancet*, **ii**, 226.

BONKOWSKY, H.L., BLOOMER, J.R., EBERT, P.S. & MAHONEY, M.J. (1975) Haem synthetase deficiency in human protoporphyria. Demonstration of the defect in liver and cultured skin fibroblasts. *Journal of Clinical Investigation*, **56**, 1139.

BOTTOMLEY, S.S., TANAKA, M. & EVERETT, M.A. (1975) Diminished erythroid ferrochelatase activity in protoporphyria. *Journal of Laboratory and Clinical Medicine*, **86**, 126.

BRODIE, M.J., MOORE, M.R., THOMPSON, G.G., GOLDBERG, A. & HOLTI, G. (1977) Haem biosynthesis in peripheral blood in erythropoietic protoporphyria. *Clinical and Experimental Dermatology*, **2**, 381.

BRUGUERA, M., ESQUERDA, J.E., MASCARO, J.M. & PINOL, J. (1976) Erythropoietic protoporphyria. A light, electron and polarization microscopical study of the liver in three patients. *Archives of Pathology and Laboratory Medicine*, **100**, 587.

CRIPPS, D.J. & GOLDFARB, S.S. (1978) Erythropoietic protoporphyria: hepatic cirrhosis. *British Journal of Dermatology*, **98**, 349.

CRIPPS, D.J. & SCHEUER, P.J. (1965) Hepatobiliary changes in erythropoietic protoporphyria. *Archives of Pathology*, **80**, 500.

CRIPPS, D.J., GILBERT, L.A. & GOLDFARB, S.S. (1977) Erythropoietic protoporphyria: juvenile protoporphyrin hepatopathy cirrhosis and death. *Journal of Paediatrics*, **91**, 744.

DAVIDSON, D.L., BLOOMER, J.R. & KLATSKIN, G. (1973) Therapy of hepatic disease in erythropoietic protoporphyria. *Gastroenterology*, **65**, 535.

DE GOEIJ, A.F.P.M., CHRISTIANSE, K. & VAN STEVENINCK, J. (1975) Decreased haem synthetase activity in blood cells of patients with erythropoietic protoporphyria. *European Journal of Clinical Investigation*, **5**, 397.

DONALDSON, E.M., MCCALL, A.J., MAGNUS, I.A., SIMPSON, J.R., CALDWELL, R.A. & HARGREAVES, T. (1971) Erythropoietic protoporphyria: two deaths from hepatic cirrhosis. *British Journal of Dermatology*, **84**, 14.

DOSS, M., LOOK, D., HENNING, H., NAWROCKI, P., SCHMIDT, A., DOLLE, W., KORB, G., LUDERS, C.H. & STROHMEYER, C. (1972) Hepatic porphyrins and urinary porphyrins and porphyrin precursors in liver cirrhosis. *Klinische Wochenschrift*, **50**, 1025.

GSCHNAIT, F., KONRAD, K., HONIGSMANN, H., DENK, H. & WOLFF, K. (1975) Mouse model for protoporphyria. 1. The liver and hepatic protoporphyrin crystals. *Journals of Investigative Dermatology*, **65**, 290.

IWANOW, V.E., TASCHEV, T., KRASTEV, L. & BARIKOV, N. (1972) Leberschadigung bie Protoporphyria erythropoieticia. *Dermatologische Monatsschrift*, **158**, 806.

KLATSKIN, G. & BLOOMER, J.R. (1974) Birefringence of hepatic pigment deposits in erythropoietic protoporphyria: specificity and sensitivity of polarization microscopy in the identification of protoporphyrin deposits. *Gastroenterology*, **67**, 295.

LANGHOF, H., HEILMEYER, L., CLOTTEN, R. & RIETSCHEL, L. (1964) Die erythropoietische Protoporphyria. *Deutsche Medizinische Wochenschrift*, **89**, 1289.

MACDONALD, D.M. & NICHOLSON, D.C. (1976) Erythropoietic protoporphyria: hepatic implications. *British Journal of Dermatology*, **95**, 157.

MAGNUS, I.A., PORTER, A.D., MCCREE, K.J., MORELAND, J.D. & WRIGHT, W.D. (1959) A monochromator—an apparatus for the investigation of the responses of the skin to ultraviolet, visible and near infra-red radiation. *British Journal of Dermatology*, **71**, 261.

MAGNUS, I.A., JARRETT, A., PRANKERD, T.A.J. & RIMINGTON, C. (1961) Erythropoietic protoporphyria. A new porphyria syndrome with solar urticaria due to protoporphyrinaemia. *Lancet*, **ii**, 448.

MASCARO, J.M., PINOL AGUADE, J., BRUGUERA, M. & GALY MASCARO, C. (1971) Histopatologie del higado en la protoporfiria eritropoyetica. *Medicina Cutanea*, **6**, 441–448.

MATILLA, A. & MOLLAND, E.A. (1974) A light and electron microscope study of the liver in a case of erythrohepatic protoporphyria and in griseofulvin induced porphyria in mice. *Journal of Clinical Pathology*, **27**, 698.

MEFFERT, H., BARTHELMES, H., SCHMIDT, N. & SONNICHSEN, H. (1974) Leberbeilung erythropoietischer Protoporphyrie. *Dermatologische Monatsschrift*, **160**, 748.

MEYER, U.A., FAH, P., KNOBLAUCH, M. & FRICK, P. (1975) Protoporphyrin hepatopathy: rare complications of erythropoietic protoporphyria or separate disease (abstract). *Porphyrins in Human Disease*. Proceedings of the International Porphyrin Meeting, Frieburg.

PERROT, H. & ORTONNE, J.P. (1978) Hyperpigmentation after bleomycin therapy. Ultrastructural study. *Archives of Dermatological Research*, **261**, 245.

REDEKER, A.G., BRONOW, R.S. & STERLING, R.E. (1963) Erythropoietic protoporphyria. *South African Journal of Laboratory and Clinical Medicine*, **9**, 235.

RYAN, E.A. (1966) Histochemistry of the skin in erythropoietic protoporphyria. *British Journal of Dermatology*, **78**, 501.

SCHMIDT, D. & STICH, W. (1971) Erythropoietische Protoporphyrie mit Porphyrinurie. Untersuchungen zur Frage der Leberbeteiligung bei erythropoietischer Protoporphyrie. *Blut*, **22**, 202.

SCOTT, A.J., ANSFORD, A.J., WEBSTER, B.H. & STRINGER, H.C.W. (1973) Erythropoietic protoporphyria with features of a sideroblastic anaemia terminating in liver failure. *American Journal of Medicine*, **54**, 251.

THOMPSON, R.P.H., MOLLAND, E.A., NICHOLSON, D.C. & GRAY, C.H. (1973) Erythropoietic protoporphyria and cirrhosis in sisters. *Gut*, **14**, 934.

WALDO, E.D. & TOBIAS, H. (1973) Needle like cytoplasmic inclusions in the liver of porphyria cutanea tarda. *Archives of Pathology*, **96**, 368.

WOLFF, K., WOLFF-SCHREINER, E. & GSCHNAIT, F. (1975) Liver inclusions in erythropoietic protoporphyria. *European Journal of Clinical Investigation*, **5**, 21.

British Journal of Dermatology (1981) **104**, 19.

Occupational argyria; light and electron microscopic studies and X-ray microanalysis

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SUMMARY

Microscopic studies have been performed on skin biopsies from five patients with occupational argyria.

Small brown-black granules were present in the dermis on light microscopy and were intensely refractile with dark-field illumination. Electron microscopy showed that the granules were electron-dense, round or oval in shape and varied in size from 30 nm to 100 nm. They were most numerous in relation to the basal lamina of the eccrine sweat glands, but were also present in relation to the basal lamina of the epidermis and dermal elastic fibres. X-ray microanalysis confirmed that many of the granules contained silver and sulphur. However, selenium, mercury, titanium and iron were also identified and it is probable that these elements were deposited in the skin also as a result of occupational exposure.

For several hundred years Sheffield has been a main centre for the refining of silver and the manufacture of silver articles. During the last 2 years we have seen five patients with generalized argyria. All were employed as furnace men in the same melting shop producing silver from crude ore. Light and electron microscopic studies, including X-ray microanalysis, have been performed on skin biopsies from these patients and this paper reports our findings.

PATIENTS AND METHODS

All five patients were male and had been employed at the factory for more than 12 years (Table 1). Clinical examination showed a striking slate-grey hyperpigmentation of the skin particularly in the sun-exposed areas of the face and neck. Two of the patients also showed a slate-blue colouration of the proximal half of their finger nails. The patients had no other medical complaint apart from the cosmetic disability of their skin.

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TABLE I. Clinical details of patients

Patient	Age (years)	Occupational History	Symptoms/Signs
P.G.	41	Foreman, melting shop—17 years	Slate-grey skin face and neck. Blue finger nails
H.L.	33	Furnaceman, melting shop—16 years	Slate-grey skin on face. Blue finger nails
J.B.	44	Furnaceman, melting shop—13½ years	Slate-grey skin on face
G.A.	41	Furnaceman, melting shop—14 years. Bullion worker—4 years	Slate-grey skin on face and neck
W.M.	33	Furnaceman, melting shop—12 years. Storeman—3 years	Slate-grey skin on forehead

Skin biopsies were taken from the hyperpigmented areas on the forehead. A portion of each biopsy was fixed in formal saline and processed by standard techniques for light microscopy. Sections were examined by both bright and dark-field illumination.

Portions of the biopsies were split in 2 N sodium bromide solution and the epidermal sheets in 1 part per 1000 L-Dopa solution at 37°C for 5 h. Quantitative studies were performed on the number of melanocytes in the epidermal sheets.

In addition, portions of the biopsies were fixed in 3% glutaraldehyde, post-fixed in osmium tetroxide and embedded in araldite. Ultrathin sections were cut, stained with lead citrate and viewed on a AEI EM6B electron microscope. X-ray microanalysis was performed in a Philips EM 400 with a high tilt goniometer and an EDAX (Energy Dispersive Analysis of X-rays) system linked to an EPIC computer (Fig. 1). Sections were cut at 100 nm, mounted on copper grids and analysed unstained with an accelerating voltage of 60 kV, a 30° tilt and with a probe size of 100–200 nm.

Control skin, removed at autopsy from the forehead of four male patients aged between 40 and 50 was also studied.

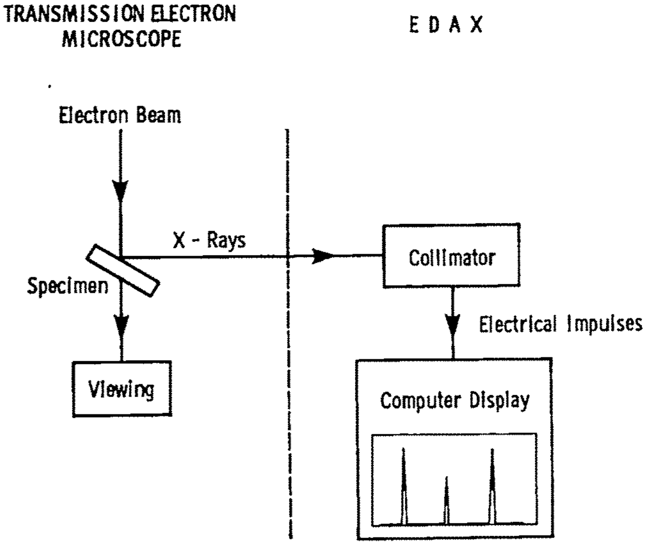

FIGURE 1. X-ray microanalysis; EDAX system used.

In an attempt to reduce the degree of skin pigmentation, two of the patients (PG and JB) were treated three times weekly for 1 year with a formulation containing 5% hydroquinone. Repeat biopsies were taken from the treated areas after 1 year.

RESULTS

Light microscopy

With bright-field illumination, small brown-black granules were identified in relation to the basal lamina of the secretory coils of the eccrine sweat glands. However, the granules were more readily visible with dark-field illumination (Fig. 2), and could also be identified in relation to the basal lamina of the epidermis, hair follicles, small blood vessels and apparently lying 'free' in the dermis. No granules were identified in the epidermis.

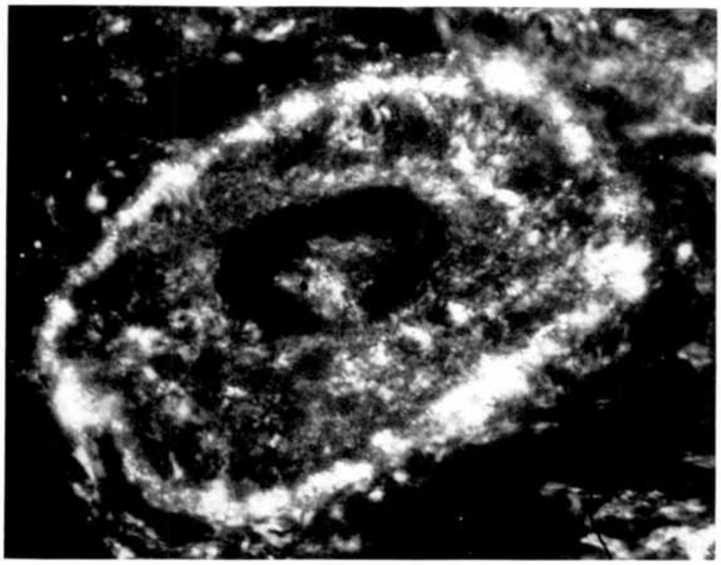

FIGURE 2. Eccrine sweat gland with refractile granules in the basal lamina. Dark-field illumination (× 950).

The granules were completely removed following treatment of the sections with sodium cyanide.

Treatment with 5% hydroquinone appeared to reduce the number of silver granules, particularly in the upper part of the dermis and around the eccrine sweat glands.

The skin from the control patients was normal.

Quantitative studies on epidermal melanocytes

The population densities of the epidermal melanocytes in the biopsies taken from these patients are shown in Table 2. The melanocytes appeared to be normal both in size and shape and their reaction to Dopa. There was a slight reduction in the number of melanocytes in the biopsies taken from the two patients following a year of treatment with 5% hydroquinone.

Electron Microscopy

In the dermis, electron-dense granules were identified which were usually round or oval in shape

P, 25, 439

TABLE 2. Melanocyte populations in epidermis before and after 1 year of treatment with hydroquinone (HQ)

		Average no. of melanocytes/mm² ± s.e. mean	
Patient	Site	Before	Following HQ treatment
P.G.	Face	2140 ± 80	1484 ± 49
H.L.	Face	1675 ± 87	—
J.B.	Face	1979 ± 65	1742 ± 67
G.A.	Face	1940 ± 62	—
W.M.	Face	2416 ± 91	—

but occasionally appeared irregular. They varied in size from 30 to 100 nm and were most numerous in relation to the basal lamina of the secretory coil of the eccrine sweat glands (Fig. 3). Smaller numbers of granules were also present in relation to the basal lamina of the epidermis (Fig. 4), small blood vessels, Schwann cells and dermal elastic and collagen fibrils. Macrophages, particularly in the papillary dermis (Fig. 5) contained numerous electron-dense bodies in lysosomal-type organelles. No granules were identified within the sweat gland epithelial cells and no abnormality was found in the epidermis.

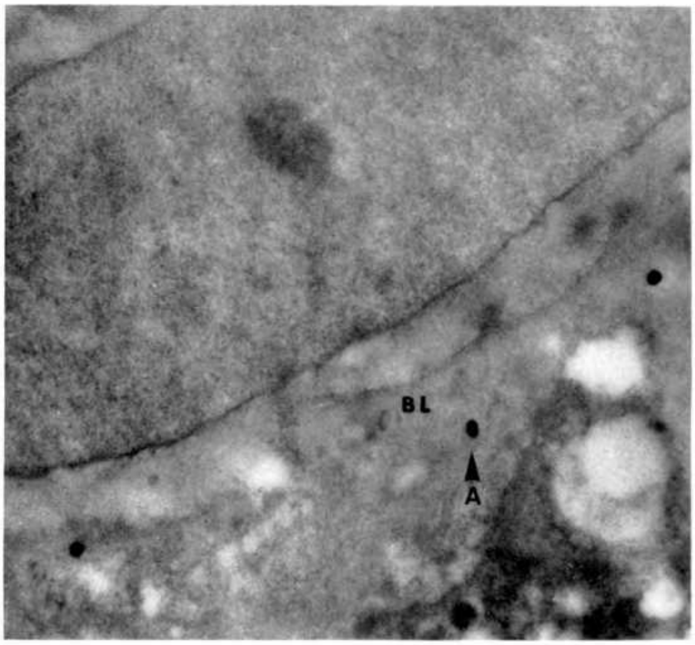

FIGURE 3. Electron micrograph showing spherical electron-dense granules (A) in relation to basal lamina (BL) of the secretory coil of an eccrine sweat gland. Unstained 100 nm section (× 27,000).

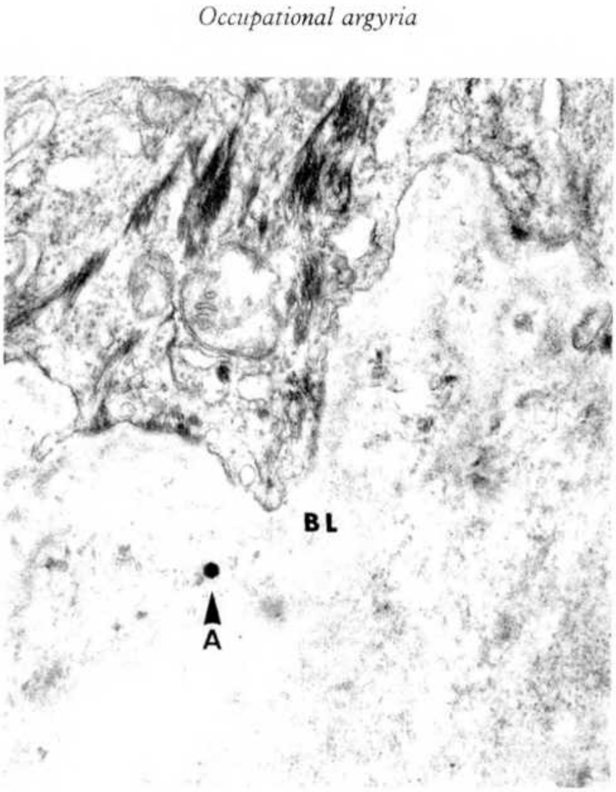

FIGURE 4. Electron micrograph of the epidermal-dermal junction zone showing a solitary electron-dense granule (A) in relation to the basal lamina (BL) which shows marked reduplication (× 40,000).

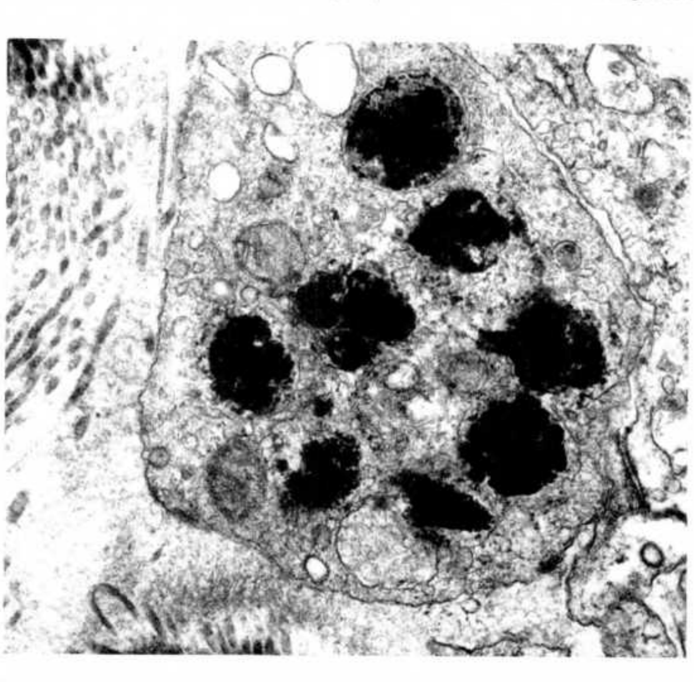

FIGURE 5. Membrane-bound aggregates of electron-dense particles in the cytoplasm of a macrophage (× 40,000).

X-ray microanalysis

X-ray microanalysis confirmed that the electron-dense granules present in relation to the basal lamina of the sweat glands, epidermis, blood vessels, elastic and collagen fibrils contained silver (Fig. 6). In addition, the same granules in all five cases contained variable amounts of sulphur and selenium. Occasional peaks were also recorded indicating the variable presence of mercury, titanium and iron in the same or different granules. Peaks due to osmium, lead and copper were recorded on the background control displays (Fig. 6). Copper was, however, absent when platinum grids were used and sulphur, despite its presence in araldite, was not registered on the background displays because of its low concentration. Metallic elements were rarely identified in the electron-dense bodies present within the macrophages. The skin from the control patients was normal.

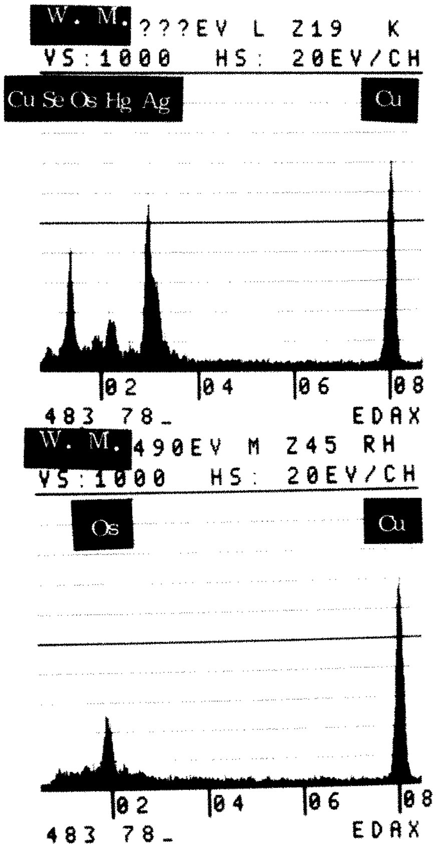

FIGURE 6. Part of X-ray microanalysis computer display. Upper-peaks due to metallic elements in electron-dense granules. Se—selenium (1·3 keV); Hg—mercury (2·19 keV); Ag—silver (2·98 keV). Lower-peaks due to background elements. Os—osmium (1·9 keV); Cu—copper (0·9 keV and 8·04 keV).

DISCUSSION

An occupational cause for generalized argyria is now rarely seen. Although at one time cases such as those presently reported were not uncommon, adequate ventilation and extraction of fumes in furnace rooms where silver refining takes place have greatly reduced the risks to workers. Occupational argyria is now more commonly seen amongst workers handling silver electroplating solutions or involved in photographic processing.

The histopathological findings in this study are similar to those previously reported (Hill & Montogomery, 1941; Prose, 1963; Mehta, Dawson-Butterworth & Woodhouse, 1966; Hönigsmann, Konrad & Wolff, 1973; Plewig, Linck & Wolff, 1977; Pariser, 1978) and confirm that the most significant abnormality is the accumulation of silver-containing granules in relation to the basement membrane of the secretory coils of the eccrine sweat glands. These findings in occupational argyria are also similar to those recently reported in patients taking various silver-containing medications (Pariser, 1978).

Proof that the granules present in our patients contained silver was provided by X-ray microanalysis. The demonstration of sulphur was not unexpected as this has been previously described and reflects the presence of silver sulphide (Hori & Miyazawa, 1977). However, the deposition of selenium, mercury, titanium and iron in patients with argyria has not been previously reported. It appears probable that our patients were exposed to the fumes of these metals while involved in the refining of the crude silver-containing ores. The ultrastructural localization of these additional elements was the same as silver and it is of interest that a similar localization has been described in a case of mercury pigmentation (Burge & Winkelmann, 1970). An ultrastructural and analytical electron microscopic study of a case of mercury pigmentation due to industrial exposure (Kennedy *et al.*, 1977) found electron-dense 400–900 nm aggregates of 12 nm particles in dermal macrophages and free in the dermis. These particles contained not only mercury but selenium as well.

No silver was identified in the epidermis and it appears unlikely that in agryria, the skin acts as an excretory organ as has been suggested previously (Buckley & Terhaar, 1973).

In our study, measurements of total body silver content or tissue silver concentrations were not made. However, it has been estimated that as little as 4–5 g of silver can produce the clinical picture of argyria (Siemun & Stolp, 1968). Neutron activation analysis has demonstrated the presence of small amounts of silver in the skin, bone and liver of people without a history of known silver intake or exposure, and it is possible that such silver may be derived from such sources as cooking utensils (Czitober, Frischauf & Leodolter, 1970).

At the present time, there is no completely effective and safe treatment for the removal of silver from the skin. Patients with argyria have a considerable cosmetic disability and though some improvement can be obtained using a depigmentory preparation, as in our two cases, the results are not entirely satisfactory.

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REFERENCES

Buckley, W.B. & Terhaar, C.J. (1973) The skin as an excretory organ in argyria. *Transactions of the St John's Hospital Dermatological Society*, **59**, 39.

Burge, K.M. & Winkelmann, R.K. (1970) Mercury pigmentation: an electronmicroscopic study. *Archives of Dermatology*, **102**, 51.

Czitober, H., Frischauf, H. & Leodolter, I. (1970) Quantitative untersuchungen bei universeller argyrose mittels neutonenaktivierungsanalyse. *Virchows Archiv. A.*, **350**, 44.

Hill, W.R. & Montgomery, H. (1941) Argyria: with special reference to the cutaneous histopathology. *Archives of Dermatology*, **44**, 588.

Hönigsmann, H., Konrad, K. & Wolff, K. (1973) Argyrose. Histologie and ultrastruktur. *Hautarzt*, **24**, 24.

Hori, Y. & Miyazawa, S. (1977) Argyria: electron microscopy and X-ray microanalysis. *Journal of Electron Microscopy*, **26**, 193.

Kennedy, C., Molland, E.A., Henderson, W.J. & Whiteley, A.M. (1977) Mercury pigmentation from industrial exposure: an ultrastructural and analytical electron microscopic study. *British Journal of Dermatology*, **96**, 367.

Mehta, A.C., Dawson-Butterworth, K. & Woodhouse, M.A. (1966) Argyria. Electron microscopic study of a case. *British Journal of Dermatology*, **78**, 175.

Pariser, R.J. (1978) Generalised argyria. Clinicopathologic features and histochemical studies. *Archives of Dermatology*, **114**, 373.

Plewig, G., Lincke, H. & Wolff, H.H. (1977) Silver blue nails. *Acta Dermato-venereologica*, **57**, 413.

Prose, P.H. (1963) An electron microscopy study of human generalized argyria. *American Journal of Pathology*, **42**, 293.

Siemund, J. & Stolp, A. (1968) Argyrose. *Zeitschrift für Hautkrankheiten*, **43**, 71.

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The effect of placebo and 'aggro' on the weal and flare response to histamine

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SUMMARY

No change in the weal and flare response to histamine was found after placebo and 'aggro', the former given double-blind, the latter in a blind study. Placebo and 'aggro' actions may involve mechanisms other than the final response to mediators of inflammation.

The weal and flare response to intradermal histamine is a reproducible and measurable vascular phenomenon and we therefore used it to study the ameliorating action of placebos (which many believe to occur) and to see whether the converse or 'aggro' effect could be elicited.

MATERIALS AND METHODS

Placebo study

Twelve male and eight female normal volunteers aged from 20 to 51 years (mostly 20–30 years) were studied. All agreed to take part in a comparative study of azatadine and placebo and they were told they would be given either the H_1 blocker or an inert compound (placebo). Tablets containing active and inert material were indistinguishable and both were given double blind. The dose response to intradermal histamine was measured before and 120 min after ingestion of 1 mg azatadine, or a tablet similar in appearance but without active material. The histamine was injected into the flexor aspect of the forearm as the acid phosphate in 0·1, 1·0 and 10 μg doses of histamine base in 0·05 ml volumes of 0·9% NaCl using a tuberculin syringe and a 16 by 0·5 mm needle. Flare diameter was measured at 5 min, weal diameter at 10 min and thickness at 20 min, which are the optimum times for their measurement (Cook & Shuster, 1980).

Aggro study

Six male and three female normal volunteers aged from 20 to 41 years, a few of whom had taken part in the placebo study, were included. They were told that the influence of a new compound thought to have histamine agonist properties was being studied and it was therefore likely that there would be some worsening of the weal and flare response to intradermal histamine. The inert tablets used

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were the same as had previously been used for the placebo study. The weal respone to i.d. histamine was measured before and 120 min after the inert tablet.

Since the placebo and 'aggro' experiments were separated by an interval of almost a year comparisons can be made only within each study using the separate pre-treatment dose-response curves for each.

RESULTS

There were small insignificant differences in erythema and wealing with placebo, 'aggro' and azatadine when compared with the corresponding pre-treatment values (Fig. 1).

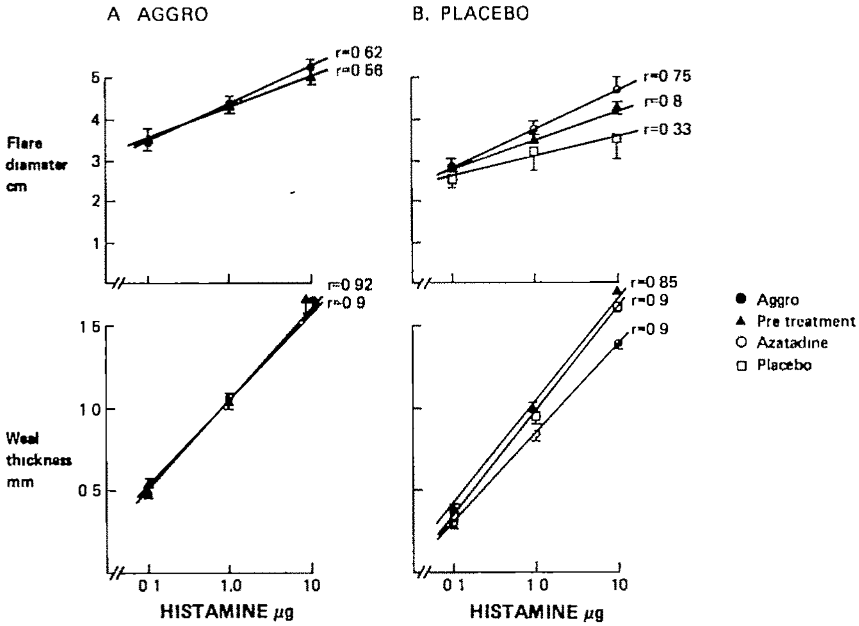

FIGURE 1. Dose-response of flare and weal. Linear regressions are shown and the respective correlation coefficients are given. Mean ± s.e. mean are plotted for each dose.

DISCUSSION

Despite our expectations we did not find a decrease in weal and flare after placebo or an increase after 'aggro'. However, weal suppression was only of the order of 14% after 1 mg azatadine and 23% after 4 mg chlorpheniramine (unpublished) which accords with the general finding that the effect of H_1 blockers on wealing is less than might be expected from their clinical activity (Bain, 1951). Thus if this poor response to active agents sets the limit to what may be expected of placebo and 'aggro', many more observations would be required to establish the biological significance of the small changes which occurred. A number of other facts could explain the absence of placebo and 'aggro' effect. Thus the double-blind design of the placebo study was more concerned with the prevention of bias than the expression of an ameliorating placebo activity, whilst the 'aggro' study, in allowing a more direct suggestion of 'aggro', did not preclude bias. A related factor may have been a poverty of suggestibility either because we are poor at its induction or because our healthy subjects were less suggestible than patients. More studies in suggestible subjects and patients who weal easily would be worthwhile. Likewise the suggestion of expected amelioration or worsening could be given by a physician more skilled in the art of placebology than we are. It may also be that placebo and 'aggro' modulate histamine release rather than effect, an action testable by the effect on dose-response of antigen and histamine liberators. Finally, it may be that the reason for the absence of objective effects is that the site of action of placebo and 'aggro' is primarily central (psychological) by altering appreciation rather than by a secondary peripheral action which modulates the weal response; this distinction will require correlation of objective and subjective responses. We conclude that if placebo and 'aggro' affect wealing responses such as that due to histamine they do so at a level before the final response to the inflammatory mediator.

ACKNOWLEDGMENTS

We are grateful to Schering Corporation for supplies of azatadine and inert tablets.

REFERENCES

COOK, L.J. & SHUSTER, S. (1980) Histamine weal formation and absorption in man. *British Journal of Pharmacology*, **69**, 579.

BAIN, W.A. (1951) Evaluation of drugs in man with special reference to antihistamines. *Analyst*, 573–579.

British Journal of Dermatology (1981) **104**, 31.

Evaluation of acid α-naphthyl acetate esterase activity as a marker of T lymphocytes in benign and neoplastic cutaneous infiltrates

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SUMMARY

Activity of the enzyme acid α-naphthyl acetate esterase (ANAE) in T lymphocytes and histiocytes in cutaneous tissue sections of patients with histologically confirmed lichen planus, chronic discoid eczema and mycosis fungoides was compared with results obtained using an *in situ* immunohistochemical technique employing an antiserum against the human T lymphocyte surface antigen (HTLA). In lichen planus and chronic discoid eczema, most of the cells in the cutaneous infiltrate exhibited both positive ANAE activity and the presence of HTLA. In mycosis fungoides, cells identified as T lymphocytes by the presence of HTLA often showed no ANAE activity. ANAE activity appears to be a useful marker of T lymphocytes in benign cutaneous infiltrates but is less reliable in labelling abnormal T cells.

Various techniques are now available for identifying T lymphocytes *in situ* in tissue sections. The use of specific antisera against the human T lymphocyte antigen HTLA (Smith *et al.*, 1973) has enabled T cells to be identified using immunofluorescence or immunoperoxidase techniques on cutaneous tissue sections in light microscopy (Lamelin *et al.*, 1978; Chu & MacDonald, 1979; Schmitt, Alario & Thivolet, 1979) and ultrastructurally (MacDonald *et al.*, 1978; Chu, Morgan & MacDonald, 1980). The use of enzyme histochemistry in mononuclear cell identification has been extensively investigated (Chichoki, Astaldi & Lisiewicz, 1972; Astaldi *et al.*, 1973; Burg, 1976) and although authors have expressed great enthusiasm for enzyme markers (Barr & Perry, 1976; Tamaoki & Essner, 1969) their results have not been totally consistent. The usefulness of ANAE as a marker of T lymphocytes has been stressed by Mueller *et al.* (1975). In this study we have assessed the value of ANAE activity for the identification of T lymphocytes in benign and malignant dermatoses.

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MATERIALS AND METHODS

Skin biopsies of representative lesions from two patients with lichen planus, three patients with chronic discoid eczema and seven patients with mycosis fungoides were obtained under local anaesthesia. The diagnosis in each patient was confirmed histologically. The biopsies were divided into two parts: (1) half was snap frozen in n-hexane and carbon dioxide (Chayen *et al.*, 1969) and stored at -70^0C; (2) half was fixed in buffered formal sucrose for 22 h, washed in Holt's gum sucrose at 4^0C for 22 h and then snap frozen in n-hexane and carbon dioxide and stored at -70^0C.

Fixative. Buffered formal sucrose pH 6·8.

Wash. Holt's gum sucrose.

Stain. Stain I: 40 ml 0·067 M Phosphate buffer pH5·0; 2·4 ml hexazotized pararosaniline; 10 mg naphthyl acetate in 0·4 ml acetone; adjust pH to 5·8 with 2N NaOH. Stain II: 1 g methyl green; 0·1 M acetate buffer pH 4·2, 100 ml.

Antisera. Rabbit anti-HTLA (Institut Merieux). This antiserum was prepared in rabbits and its specificity controlled by microlymphocytotoxicity and rosette inhibition tests (Touraine *et al.*, 1974). It was used at a dilution of 1 in 2 in phosphate buffered saline (PBS). Sheep antirabbit Fab Po (Institut Pasteur): this was used at a dilution of 1 in 30 in PBS. Sheep anti-mouse Fab Po (Institut Pasteur): this was used as a control serum at a dilution of 1 in 30 in PBS.

4μ cryostat sections of the unfixed frozen tissue (1) were cut on to clean glass slides and fixed for 10 min in 2% paraformaldehyde. The sections were then subjected to an indirect immunoperoxidase reaction as previously described (Chu & MacDonald, 1979). The sections were finally washed in PBS, dehydrated in graduated alcohols, bathed in xylene and mounted in DPX.

10 μ cryostat sections of the fixed frozen tissue (2) were cut on to glass slides which had been pretreated with formal gelatin and allowed to dry for 1 h. The sections were then stained with stain I for 3 h at room temperature in a humidity chamber, washed in distilled water and subsequently counterstained with stain II for 2 min. The sections were finally washed in distilled water, dehydrated through graduated alcohols, bathed in xylene and mounted in DPX.

RESULTS

In the 4μ sections, T lymphocytes could easily be identified by the presence of a fine brown granular deposit of peroxidase reaction product on the cytoplasmic membrane of the cells. In lichen planus, chronic discoid eczema and mycosis fungoides, most cells comprising the cutaneous infiltrates were shown to be T lymphocytes (Fig. 1). Control sections showed no positive cytoplasmic labelling.

In the 10μ sections ANAE activity was demonstrated by a red-brown reaction product within the cells. Two patterns of ANAE activity were present. The first was the so-called M-like diffuse staining of the entire cytoplasm (Obrist, Albrecht & Nagel, 1978) representing cells of the histiocyte series The second was a granular staining comprising 1–3 granules of 0·5–2 μ diameter in the peripheral part of the cytoplasm, representing the T-like pattern of T lymphocytes.

In lichen planus and chronic discoid eczema, the majority of the cells in the dermal infiltrate exhibited T-like staining. A varying number of macrophages were also present (Fig. 2).

In mycosis fungoides, however, results were not as consistent. In two patients the majority of cells in the dermal and epidermal infiltrates showed a T-like pattern. In the other five cases, however, only 20–50% of the cells in the cutaneous infiltrates exhibited T-like staining. In these patients, macrophages were easily identified but many of the infiltrating cells demonstrated no ANAE activity (Fig. 3).

Accurate counting of T lymphocytes using either of these techniques was difficult and only a

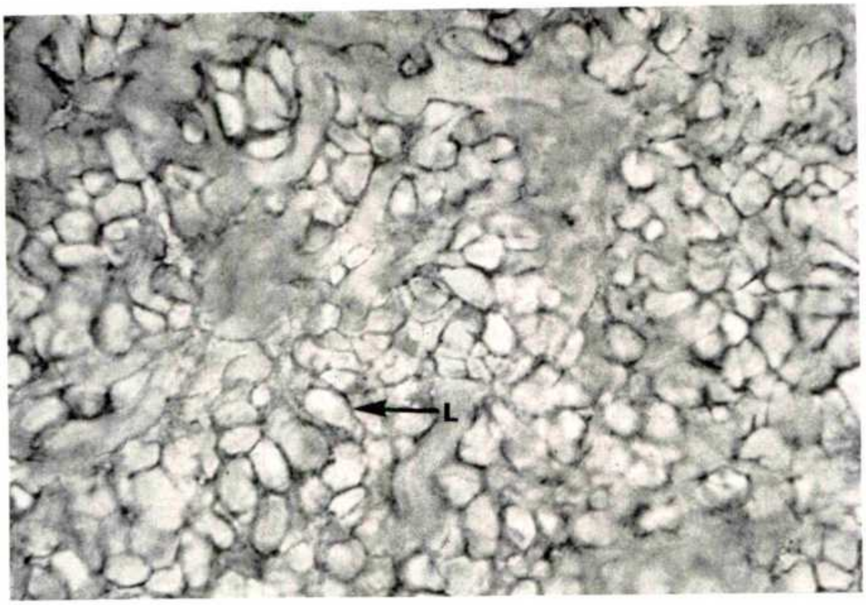

FIGURE 1. Mycosis fungoides (×640). Dermal infiltrate of predominantly T lymphocytes. L—peripheral positive labelling of T lymphocyte by peroxidase reaction product.

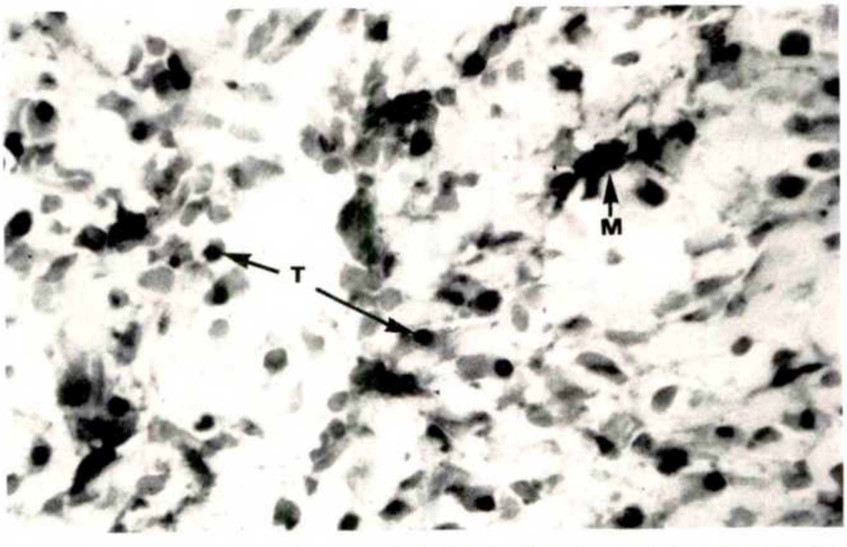

FIGURE 2. Lichen planus (× 300). Dermal infiltrate. M—macrophages exhibiting intensive diffuse cytoplasmic ANAE activity; T—T lymphocytes demonstrating positive granular ANAE activity.

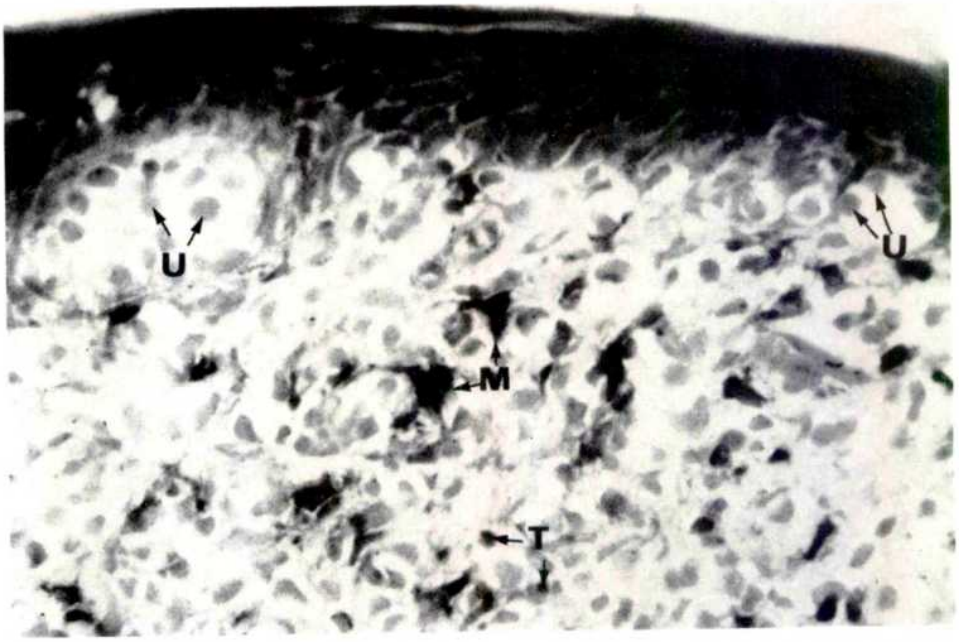

FIGURE 3. Mycosis fungoides (× 120). U—lymphocytes in Pautrier microabscesses showing no ANAE activity; T—T lymphocytes; M—macrophages.

general impression of T cells present could be gained. In the ANAE sections enumeration w particularly difficult when a large number of histiocytes were present as the diffuse staining of the cells tended to mask the finer granular staining of T lymphocytes.

COMMENT

Mueller *et al.* (1975) using a modified technique to demonstrate ANAE activity in mouse lymphocyt revealed a population of lymphocytes which exhibited a dot like activity of this enzyme and whi corresponded to the anatomical distribution of T lymphocytes. They also showed that easily detectab esterase activity was acquired by T cells before, during and just after release into the circulation, b was not present in T cell precursors.

Ranki, Totterman & Hayry (1976a; 1976b) and Totterman, Ranki & Hayry (1977) demonstrat in murine and human cells that the ANAE marker was lost by some T lymphocytes after stimulati by phytohaemagglutinin (PHA) and in the mixed lymphocyte culture (MLC) but was retained af stimulation by concanavalin A. Thus the ANAE marker was shown to be useful in detecting matu resting T lymphocytes but unreliable in labelling transformed cells.

Hovmark (1977), using ANAE staining, demonstrated the predominant T cell nature of t cellular infiltrates of various dermatoses including one patient with Sézary syndrome and one patie with poikiloderma atrophicans vasculare. The only other report of ANAE activity in abnormal T ce (Kulenkampff, Janossy & Greaves, 1977) showed faint cytoplasmic ANAE activity indicative thymocytes, but no T-like staining in four out of five patients with thymocyte surface phenoty acute lymphoblastic leukaemia.

Our results confirm that in benign dermatoses, T lymphocytes can be identified by ANAE staini

However, the ANAE marker is unreliable when abnormal T lymphocytes are examined. Many of the cells in the cutaneous infiltrates of mycosis fungoides identifiable as T cells by the presence of HTLA did not exhibit positive T-like ANAE activity.

The immunohistochemical technique is preferable to ANAE staining as a universal label of T lymphocytes. Both methods of T cell identification are technically simple and employ commercially available reagents. Results using these techniques differed principally in their consistency in labelling malignant T lymphocytes. Touraine, Hadden & Good (1977) have shown that T lymphocytes acquire HTLA at an early stage of cell maturation. The detection of HTLA would therefore be expected to be more useful in identifying cells that have undergone malignant change and possibly become less well differentiated.

ANAE activity obviously has a role to play in the investigation and identification of T lymphocytes, but we support the view of Obrist *et al.* (1978) that it is a technique to supplement rather than to replace existing techniques of lymphocyte identification.

ADDENDUM

Recently Sterry, Steigleder & Pullman (1980) have employed non-specific esterase labelling to demonstrate a predominance of T lymphocytes in cutaneous tissue sections of nine cases of mycosis fungoides and four cases of parapsoriasis-en-plaques.

REFERENCES

Astaldi, G., Lisiewicz, J., Chichoki, T. & Burlina, A. (1973) The lysosomal enzymes in lymphocytes. II. Activated lymphocytes. *Acta Vitaminologica et Enzymologica*, **27**, 197.

Barr, R.D. & Perry, S. (1976) Lysosomal acid hydrolases in human lymphocyte sub-populations. *British Journal of Haematology*, **32**, 365.

Burg, Von G. (1976) Fortschritte in der Diagnostik kutaner Lymphome. Enzymzytochemische und immunzytologische Untersuchungen. *Fortschritte der Medizin*, **94**, 1089.

Chayen, J., Bitensky, L., Butcher, R. & Poulter, L. (1969) *A guide to practical histochemistry*, p. 5. Oliver and Boyd, Edinburgh.

Chichoki, T., Astaldi, G. & Lisiewicz, J. (1972) The lysosomal enzymes in lymphocytes. 1. Normal lymphoid tissue. *Acta Vitaminologica et Enzymologica*, **26**, 3.

Chu, A.C. & MacDonald, D.M. (1979) Identification *in situ* of T lymphocytes in the dermal and epidermal infiltrates of mycosis fungoides. *British Journal of Dermatology*, **100**, 177.

Chu, A.C., Morgan, E. & MacDonald, D.M. (1980) Ultrastructural identification of T lymphocytes in tissue sections of mycosis fungoides. *Journal of Investigative Dermatology*, **74**, 17.

Hovmark, A. (1977) Acid α naphthyl acetate esterase staining of T lymphocytes in human skin. *Acta Dermato-vereneologica*, **57**, 497.

Kulenkampff, J., Janossy, G. & Greaves, M.F. (1977) Acid esterase in human lymphoid cells and leukaemic blasts: a marker for T lymphocytes. *British Journal of Haematology*, **36**, 231.

Lamelin, J.P., Thomasset, N., Andre, C., Brochier, J. & Revillard, J.P. (1978) Study of human T and B lymphocytes with heterologous antisera. III. Immunofluorescence studies on tonsil sections. *Immunology*, **35**, 463.

MacDonald, D.M., Schmitt, D., Germain, D. & Thivolet, J. (1978) Ultrastructural demonstration of T cells in cutaneous tissue sections using specific anti-human T cell antiserum. *British Journal of Dermatology*, **99**, 641.

Mueller, J., Brun del Re, C., Buerki, H., Keller, H.U., Hess, M.W. & Cottier, H. (1975) Non-specific acid esterase activity: a criterion for differentiation of T and B lymphocytes in mouse lymph nodes. *European Journal of Immunology*, **5**, 270.

Obrist, R., Albrecht, R. & Nagel, G.A. (1978) Identification of lymphocyte sub-population by simultaneous E rosette formation and unspecific acid esterase staining. *Experientia*, **34**, 661.

RANKI, A., TOTTERMAN, T.M. & HAYRY, P. (1976a) Identification of mouse T and B lymphocytes from cytocentrifuged cell smears. *Clinical and Experimental Immunology*, **26**, 632.

RANKI, A., TOTTERMAN, T.M. & HAYRY, P. (1976b) Identification of resting human T and B lymphocytes by acid α naphthyl acetate esterase staining combined with rosette formation with *Staphylococcus aureus* strain Cowan I. *Scandinavian Journal of Immunology*, **5**, 1129.

SCHMITT, D., ALARIO & THIVOLET, J. (1979) *In situ* characterization of tissue cells of cutaneous infiltrates using specific membrane antigens. *Clinical and Experimental Dermatology*, **4**, 161.

SMITH, R.W., TERRY, W.D., BUELL, D.N. & SELL, K.W. (1973) An antigenic marker for human thymic lymphocytes. *Journal of Immunology*, **110**, 884.

STERRY, W., STEIGLEDER, G.-K. & PULLMAN, H. (1980) *In situ* identification and enumeration of T-lymphocytes in cutaneous T cell lymphomas by demonstration of granular activity of acid non-specific esterase. *British Journal of Dermatology*, **103**, 67.

TAMAOKI, M. & ESSNER, E. (1969) Distribution of acid phosphatase, β glucuronidase and N-acetyl-B-glucosaminidase activities in lymphocytes of lymphatic tissue of man and rodents. *Journal of Histochemistry and Cytochemistry*, **17**, 238.

TOTTERMAN, T.H., RANKI, A. & HAYRY, P. (1977) Expression of the acid α naphthyl acetate esterase marker by activated and secondary T lymphocytes in man. *Scandinavian Journal of Immunology*, **6**, 305.

TOURAINE, J.L., HADDEN, J.W. & GOOD, R.A. (1977) Sequential stages of T lymphocyte differentiation. *Proceedings of the National Academy of Science*, **74**, 344.

TOURAINE, J.L., TOURAINE, F., KISZKISS, D.F., CHOI, Y.S. & GOOD, R.A. (1974) Heterologous specific antiserum for identification of human T lymphocytes. *Clinical and Experimental Immunology*, **16**, 503.

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Ultrastructural evidence suggesting an immunomodulatory activity of oral retinoid

ITS EFFECT ON DERMAL COMPONENTS IN PSORIASIS

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SUMMARY

The effects of the aromatic retinoid Ro 10–9359 on the fine structure of psoriatic dermis were investigated by electron microscopy in five patients after 1, 2, and 3 weeks of treatment (1 mg/kg/day). Our findings show that aromatic retinoid, additionally to its epidermal effects, exerts a distinct influence on dermal components in psoriasis. Ultrastructural evidence is provided suggesting that this drug stimulates lymphocytes and monocytes, promoting their differentiation into Sézary-like lymphocytic cells, activated macrophages and dermal Langerhans cells. The appearance of these immunocompetent cell lines may modulate the cell-mediated immune response in psoriasis, obviously reflecting a pharmacological action of the drug additional to its definite influence on epidermal keratinization.

Previous ultrastructural studies in experimental animals and in humans (Matter & Bollag, 1977; Orfanos & Runne, 1978; Tsambaos, Mahrle & Orfanos, 1980) have demonstrated that the oral aromatic retinoid Ro 10-9359 exerts a distinct influence on the differentiation and keratinization processes of epidermal tissue. The effects of this drug on the dermis, however, have remained entirely unknown. In our previous preliminary paper on the retinoid-induced epidermal alterations in psoriasis only a brief reference to some modifications of the dermal vessels was made.

The present investigation was performed in order to define the nature and the extent of the dermal changes in psoriatic skin under systemic administration of aromatic retinoid. In view of the dermo-epidermal interactions in normal and in diseased skin, such changes may have far-reaching implications in the mechanisms of action of aromatic retinoid in psoriasis and possibly in other disorders of keratinization.

Our findings show that the aromatic retinoid exerts a distinct influence on the ultrastructure of dermal cell components in psoriasis which may represent an immunomodulatory effect.

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MATERIALS AND METHODS

Skin biopsies were obtained from typical lesions of five psoriatic patients before and 1, 2 and 3 weeks after the onset of oral treatment with the aromatic retinoid Ro 10-9359* (1 mg/kg/day). The specimens were fixed in a mixture containing 4% formaldehyde and 2·5% glutaraldehyde buffered with sodium cacodylate (pH 7·2) and were postfixed in 1% osmium tetroxide solution. After dehydration in rising concentrations of acetone, the samples were embedded in Araldite. Ultrathin sections were stained with uranyl acetate and lead citrate and were examined in a Zeiss EM 9A electron microscope.

RESULTS

The morphological alterations found in psoriatic dermis after one week of retinoid administration did not differ significantly from those observed after 2 or 3 weeks; thus they will be described here together and will be compared to the ultrastructural features of psoriatic dermis before treatment.

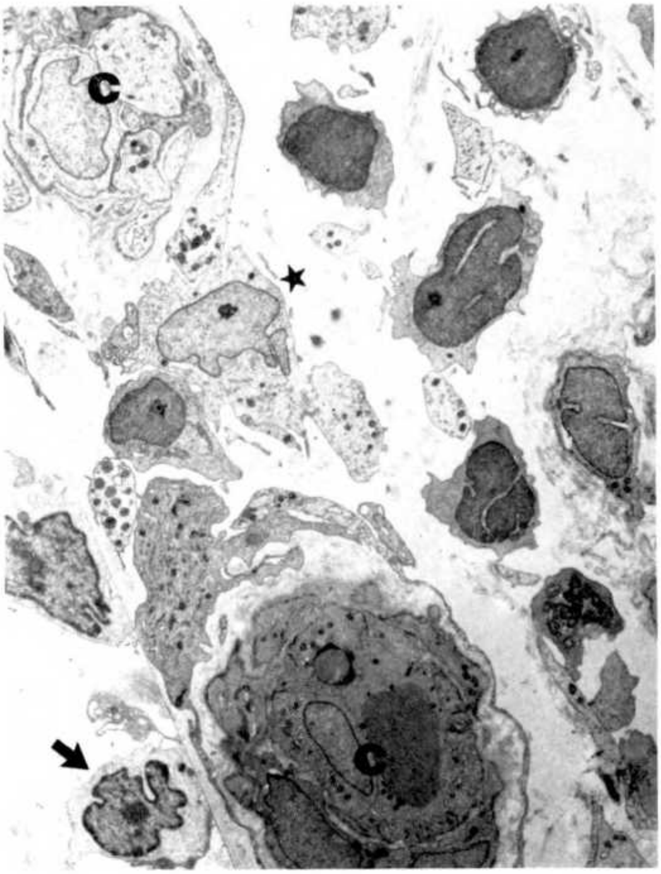

FIGURE 1. Dermal cell infiltrate in psoriatic lesion after 3 weeks of treatment with oral aromatic retinoid. In the vicinity of two dermal capillaries (c) dermal Langerhans cell (star), lymphocytes and mononuclear cells with indented nuclei are seen, resembling Sézary cells (arrow) (× 3,700).

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* The aromatic retinoid was kindly supplied by Hoffmann-La Roche AG, Grenzach-Wyhlen.

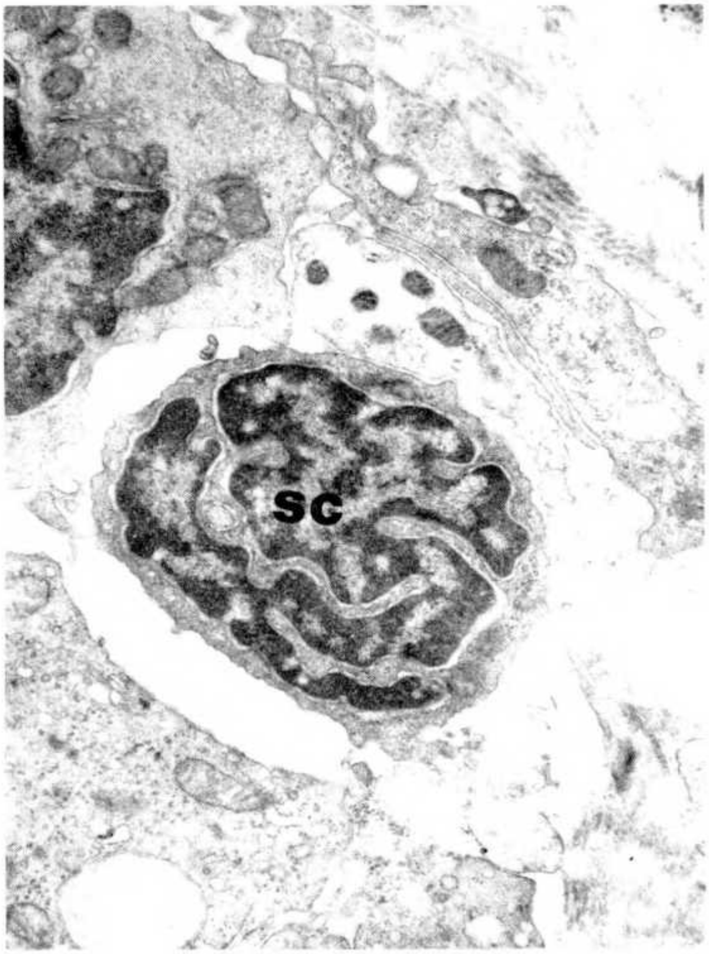

FIGURE 2. Typical Sézary cell (sc) in psoriatic dermis after 1 week of treatment with aromatic retinoid (× 14,700).

The most prominent retinoid-induced dermal change in psoriasis was seen in the inflammatory cellular infiltrate. Though its density remained practically unchanged during the 3-week administration of the drug, its cellular composition underwent important alterations. Besides normal small and medium sized lymphocytes we found numerous Sézary-like lymphocytic cells with deeply indented nuclei and cytoplasm of variable size (Fig. 1). Needless to say, lymphocytic infiltrate could also be observed before treatment in all specimens, but Sézary-like lymphocytic cells were rather rare. In addition, after treatment, large histiocytes were numerous; they showed differentiation into activated macrophages and, in places, into dermal Langerhans cells which often revealed plasma membrane contacts with lymphocytic cells (Fig 3). Most activated macrophages and Langerhans cells showed prominent Golgi membranes and endoplasmic reticulum (Fig 4). Moreover, myelin figures, autophagosomes, melanosomes, multivesiculated bodies, worm-like structures and crystalloid inclusions were seen in their cytoplasm (Fig 5). Dermal Langerhans cells were frequently found in perivascular areas, sometimes located between pericytes and endothelial cells of the vascular wall; in untreated

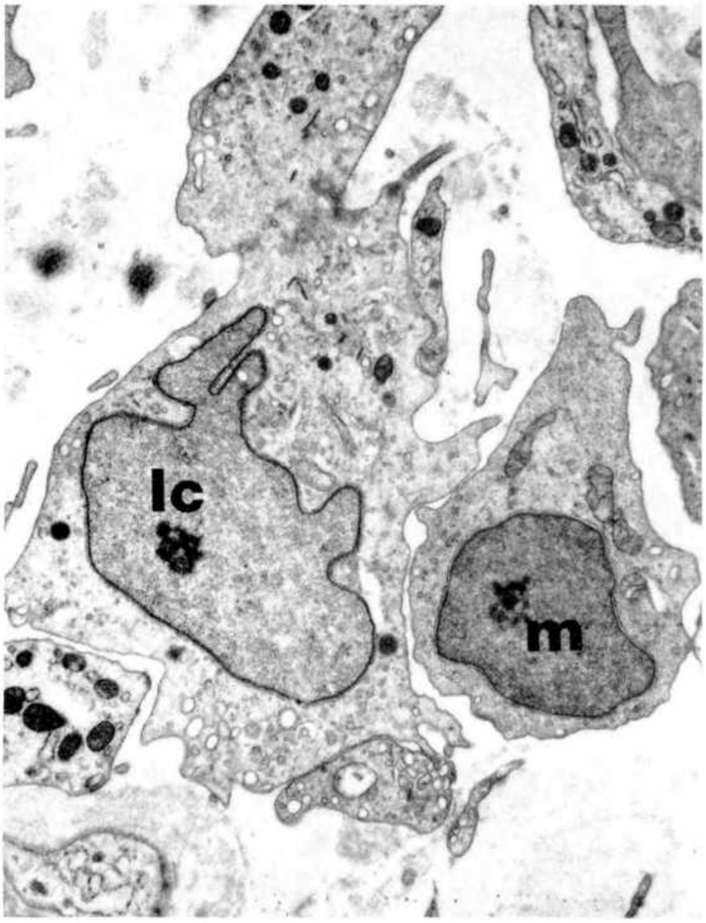

FIGURE 3. Dermal Langerhans cell (lc) in close apposition to a mononuclear cell (m) after 3 weeks of retinoid therapy (high power view from Fig. 1) (× 10,500).

psoriatic skin only one dermal Langerhans cell was detected in hundreds of examined sections and the number of activated macrophages was much lower than that after treatment.

The ultrastructural features of fibroblasts and plasma cells in the psoriatic dermis were not considered as being clearly altered under retinoid therapy; nevertheless, the number and size of their characteristic organelles and their secretory activity seemed increased overall (Fig 6). Also, the number of mast cells remained unchanged as compared to the untreated psoriatic dermis; mast cell granules, however, showed fusion of membranes and complete or partial loss of their electron density after treatment. Lipid droplets could often be observed in their cytoplasm and degranulation figures were repeatedly seen.

Collagen and elastic fibres revealed no obvious alteration in their fine structure before or after treatment with the aromatic retinoid. Finally, thorough examination of the dermal capillaries showed that vasodilatation was still obvious. Endothelial gaps and fenestrations were also still present but less frequent than in the untreated psoriatic skin. In several instances the alterations of the vascular walls were well restored and extravasation was clearly less pronounced.

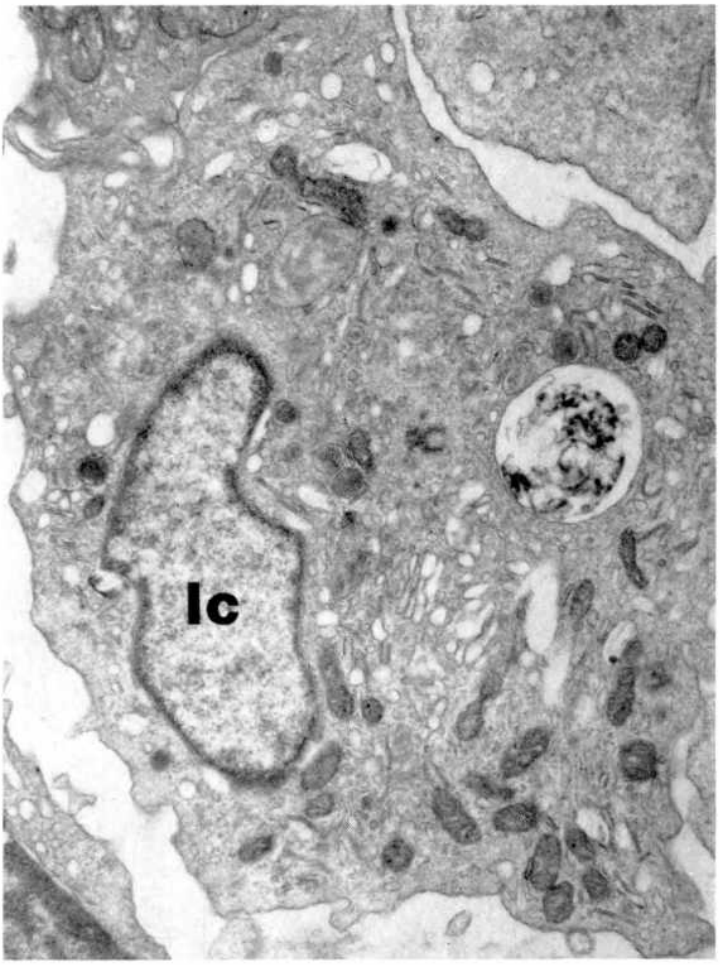

FIGURE 4. Large dermal Langerhans cell (lc) in psoriatic lesion after 2 weeks of oral retinoid treatment. Increased cytoplasmic activity and numerous Langerhans granules are seen (× 16,800).

DISCUSSION

The most prominent ultrastructural change in psoriatic dermis under treatment with the aromatic retinoid was found in the inflammatory cellular infiltrate. In contrast to its density, which remained practically unchanged, its composition was clearly altered. Sézary-like lymphocytic cells and activated macrophages were obviously increased as compared to the untreated psoriatic skin, and dermal Langerhans cells with prominent cytoplasmic activity appeared. Corresponding studies on psoriatic skin treated topically with dithranol revealed that after three weeks of treatment the dermal infiltrate was less pronounced than in the pre-treatment phase and failed to demonstrate any significant change in its cellular composition (unpublished data).

Sézary-like lymphocytic cells may occur in normal epithelial tissue and are considered to originate from lymphocytes; they are probably of T-cell nature since they reveal membrane receptors for uncoated sheep erythrocytes (Gupta, Safai & Good, 1979). Increased numbers of such Sézary-like cells

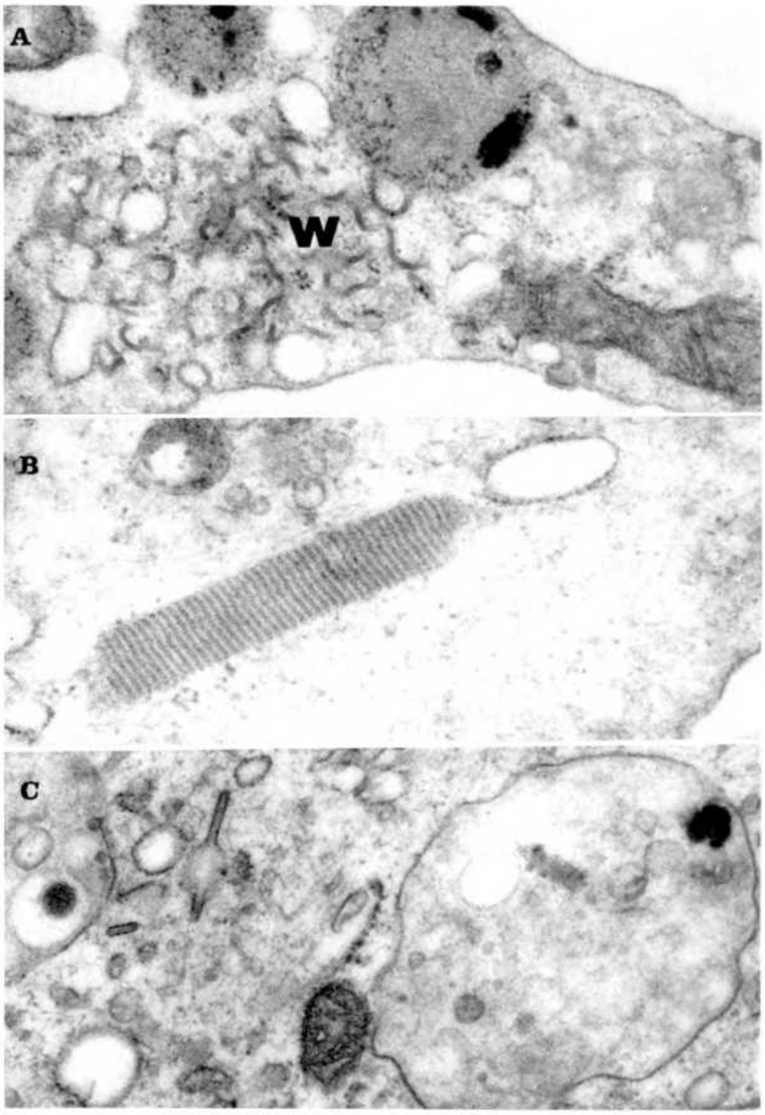

FIGURE 5. (A) Worm-like structures (W), cytoplasmic vesicles and autophagosomes in an activated macrophage after 1 week of oral retinoid treatment. (B) Cytoplasmic crystalloid inclusion within an activated macrophage after 1 week of therapy. (C) Cytoplasmic inclusions and vacuoles in a stimulated dermal Langerhans cell after 2 weeks of treatment (× 39,000).

have been observed in oral mucosa in mice subsequent to antigenic challenge (Burkhardt *et al.*, 1979). In human dermis Sézary-like lymphocytic cells occur under pathological conditions, particularly in those with concomitant antigenic persistence, such as mycosis fungoides. They occasionally occur in psoriatic dermis before treatment (Flaxman, Zelazny & van Scott, 1971) and were also described in psoriatic epidermis after local treatment with retinoic acid (Orfanos *et al.*, 1973).

The increase of these cells in psoriatic dermis after oral administration of aromatic retinoid, as shown in the present study, may represent an enhanced differentiation of lymphocytes into this cell

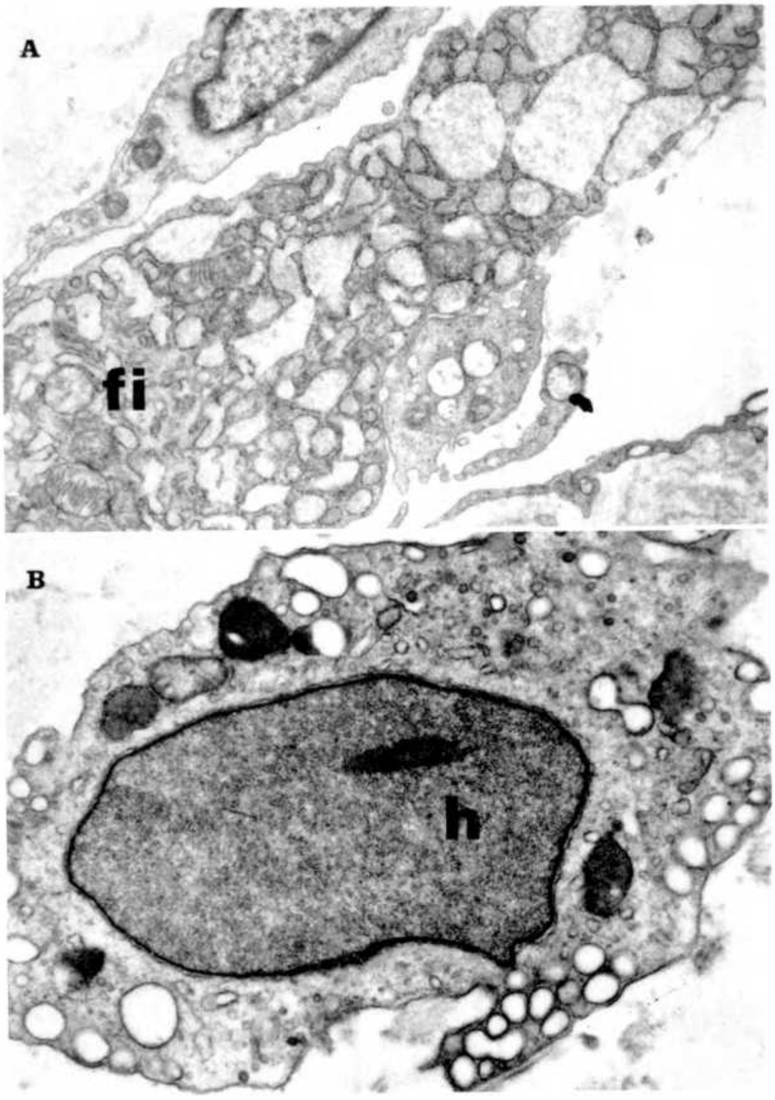

FIGURE 6. (A) Stimulated fibroblast (fi) with prominent endoplasmic reticulum containing large amounts of an amorphous material after 1 week of retinoid administration (× 10,500). (B) Unclassified histiocytic (h) cell with distinct cytoplasmic activity showing vacuoles and phagolysosomes after 3 weeks of therapy (× 12,600).

line due to (a) a direct stimulatory effect of the drug or (b) the influence of some antigenic changes induced by aromatic retinoid in the microenvironment of lymphocytes. Indeed, changes of the antigenic determinant characteristics were described in tumour cells after treatment with retinoic acid (Solter *et al.*, 1979).

For a long time the origin and the function of skin Langerhans cells have remained an unresolved enigma. Recently it has been shown that Langerhans cells are special macrophages (Rowden, Phillips & Lewis, 1979a) being most likely immunocompetent cells derived from a mobile cell pool, which originates in the bone marrow (Katz, Tamaki & Sachs, 1979). Monocytes seem to be their precursors since Langerhans cells possess surface markers of the monocyte-histiocyte-macrophage series expressing Fc-IgG and C_3 receptors (Stingl *et al.*, 1977) and Ia antigens (Rowden *et al.*, 1979b; Frelinger *et al.*, 1979). Furthermore, Langerhans cells circulating between epidermis, dermis and vessels can obviously function both as stimulators in a mixed lymphocyte reaction and as antigen-presenting cells (Stingl *et al.*, 1978). Increased numbers of Langerhans cells have been reported in the human and animal dermis in contact allergic hypersensitivity reactions (Silberberg, Baer & Rosenthal, 1976).

The increased numbers of dermal Langerhans cells and activated macrophages in psoriasis under treatment with aromatic retinoid suggest that this drug may be capable of stimulating the monocytes to further differentiation in tissue. This hypothesis is consistent with the statistically significant trend of monocytes to increase in the peripheral blood of retinoid-treated psoriatic patients (Orfanos *et al.*, 1979). Moreover, it is supported by the accelerating effect of retinoids on allogeneic graft rejection (Mayer *et al.*, 1978), in which Langerhans cells are considered to have an important role (Katz *et al.*, 1979).

The results of our investigation indicate that aromatic retinoid, in addition to its action on epidermal cells, exerts a distinct influence on dermal cell components in psoriasis. Only minor other tissue changes were found. In particular, the data presented here, though purely morphological, suggest that aromatic retinoid may stimulate lymphocytes and monocytes, promoting their differentiation into immunocompetent cell lines and, therefore, may modulate the immune response in the diseased skin. In recent years there has been growing evidence that some defect of the immune-inflammatory cell function including T-lymphocytes may occur in psoriasis (Krueger, Hill & Jederberg, 1978; Glinski *et al.*, 1978); however, a possible relationship between these abnormalities and the pathogenesis of epidermal hyperproliferation in this disease is far from clearly understood.

It remains to be elucidated, therefore, whether the therapeutic action of retinoid is due to its definite influence on epidermal keratinization (Orfanos & Runne, 1978), or to its immunomodulatory influence on dermal cell components, or to both. The last possibility seems not unlikely; immunostimulatory properties have also been ascribed to the parent substance of aromatic retinoid, namely to vitamin A itself.

REFERENCES

BURKHARDT, A., ROS, I.R., LONING, T., GEBBERS, J.O., OTTO, H.F. & SEIFERT, G. (1979) Interepithelial cells of the oral mucosa in mice. An ultrastructural classification with reflections on the origin of the Langerhans cell. *Virchows Arch. A. Path. Anat. and Histol*, **384**, 223

FLAXMAN, B.A., ZELAZNY, G. & VAN SCOTT, E.J. (1971) Nonspecificity of characteristic cells in mycosis fungoides. *Archives of Dermatology*, **104**, 141.

FRELINGER, J.G., HOOD, L., HILL, S. & FRELINGER, J.A. (1979) Mouse epidermal Ia molecules have a bone marrow origin. *Nature*, **282**, 321.

GLINSKI, W., OBALEK, S., LANGNER, A., JABLONSKA, S. & HAFTED, M. (1978) Defective function of T lymphocytes in psoriasis. *Journal of Investigative Dermatology*, **70**, 105.

GUPTA, S., SAFAI, B. & GOOD, R.A. (1978) Subpopulations of human T lymphocytes. IV. Quantitation and distribution in patients with mycosis fungoides and Sézary syndrome. *Cellular Immunology*, **39**, 18.

KATZ, S.I., TAMAKI, K. & SACHS, D.H. (1979) Epidermal Langerhans cells are derived from cells originating in bone marrow. *Nature*, **282**, 324.

KRUEGER, G.G., HILL, H.R. & JEDERBERG, W.W. (1978) Inflammatory and immune cell function in psoriasis–a subtle disorder. I. *In vivo* and *in vitro* survey. *Journal of Investigative Dermatology*, **71**, 189.

MATTER, A. & BOLLAG, W. (1977) A fine structural study on the therapeutic effect of an aromatic retinoid on chemically induced skin papillomas of the mouse. *European Journal of Cancer*, **13**, 831.

MAYER, H., BOLLAG, W., HÄNNI, R. & RÜEGG, R. (1978) Retinoids, a new class of compounds with prophylactic and therapeutic activities in oncology and dermatology. *Experientia*, **34**, 1105.

ORFANOS, C.E., MAHRLE, G., GOERZ, G., HAPPLE, R., HOFBAUER, M., LANDES, E. & SCHIMPF, A. (1979) Laboratory investigations in patients with generalized psoriasis under oral retinoid treatment. A multicenter study of computerized data. *Dermatologica*, **159**, 62.

ORFANOS, C.E. & RUNNE, U. (1978) Tissue changes after oral administration of retinoid. *Dermatologica*, **157**, (Suppl.) 19.

ORFANOS, C.E., SCHMIDT, H.W., MAHRLE, G., GARTMANN, H. & LEVER, W.F. (1973) Retinoic acid in psoriasis: its value for topical therapy with and without corticosteroids. *British Journal of Dermatology*, **88**, 167.

ROWDEN, G., PHILLIPS, T.M. & LEWIS, M.G. (1979a) Ia antigens on indeterminate cells of the epidermis: immunoelectron microscopic studies of surface antigens. *British Journal of Dermatology*, **100**, 531.

ROWDEN, G., PHILLIPS, T.M., LEWIS, M.G. & WILKINSON, R.D. (1979b) Target role of Langerhans cells in mycosis fungoides: transmission and immuno-electron microscopic studies. *Journal of Cutaneous Pathology*, **6**, 364.

SILBERBERG, I., BAER, R.L. & ROSENTHAL, S.A. (1967) The role of Langerhans cells in allergic contact hypersensitivity. A review of findings in man and guinea pigs. *Journal of Investigative Dermatology*, **66**, 210.

SOLTER, D., SHEVINSKY, L., KNOWLES, B.B. & STRICKLAND, S. (1979) The induction of antigenic changes in a teratocarcinoma stem cell line (F9) by retinoic acid. *Developmental Biology*, **70**, 515.

STINGL, G., KATZ, C.I., CLEMENT, L., GREEN, I., SHEVACH, E.M. (1978) Immunologic functions of Ia-bearing epidermal Langerhans cells. *Journal of Immunology*, **121**, 2005.

STINGL, G., WOLFF-SCHREINER, E.C., PICHLER, W., GSCHNAIT, F., KNAPP, W. & WOLFF, K. (1977) Epidermal Langerhans cells bear Fc and C_3 receptors. *Nature*, **268**, 245.

TSAMBAOS, D., MAHRLE, G. & ORFANOS, C.E. (1980) Epidermal changes induced by oral excess of aromatic retinoid in guinea pigs. *Archives of Dermatological Research*, **267**, 141.

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Plasma 8-methoxypsoralen concentrations in photochemotherapy of psoriasis

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SUMMARY

A quantitative study of 8-methoxypsoralen plasma profiles in patients with psoriasis undergoing photochemotherapy shows that a poor response to the treatment may be explained in terms of abnormal pharmacokinetic behaviour. However, not all 'poor responders' exhibit an abnormal pattern and for these an alternative explanation is required.

The successful treatment of psoriasis with photochemotherapy using oral 8-methoxypsoralen (8-MOP) and longwave ultraviolet radiation between 320 and 400 nm (UV-A) has been accompanied by a number of investigations into the pharmacokinetics of the drug (Pathak *et al.*, 1974; Thune & Volden 1977; Steiner *et al.*, 1978; Hensby, 1978; Herfst, Koot-Gronsveld & de Wolff, 1978; Wulf & Hart, 1979). The majority of 8-MOP plasma level studies have concentrated on the levels obtained 2 h after oral dosing since there is evidence to indicate that the peak of skin photosensitivity occurs around this time (Kligman & Goldstein, 1973; Pathak *et al.*, 1974). However, a limited number of studies have attempted to show the plasma profile of the drug at varying times after ingestion, revealing significant inter-individual variation, not only in peak plasma concentrations obtained, but also in the time course of the profiles (Steiner *et al.*, 1977; Thune, 1978; Busch *et al.*, 1978; Gazith, Schalla & Schaefer, 1978). No significant correlation between peak plasma levels and efficacy of treatment has been reported but in a recent study (Wagner *et al.*, 1979) plasma 8-MOP concentrations were found to be lower in a group of 'poorly responsive' subjects than in those whose response was 'adequate'. In addition, peak plasma concentrations were achieved less rapidly in the former group.

This paper reports on plasma 8-MOP pharmacokinetics in a study of patients undergoing photochemotherapy and in which a similar comparison between good and poor responders has been made.

MATERIALS AND METHODS

Patients, treatment and blood specimens

Ten patients undergoing photochemotherapy for the treatment of psoriasis provided serial blood samples over a 24-h period. The studies were done with the informed consent of the patients and the

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TABLE 1. Details of treatment in subjects with good and poor response to 8-MOP photochemotherapy

Case no.	Name	Sex	Age	Dose of 8-MOP (mg)	Response to treatment	Duration of treatment (weeks)	Total irradiation (J/cm^2)
1	J.C.	M	47	50	Good	8	70·0
2	G.D.	M	51	40	Good	4	30 0
3	J.N.	F	51	50	Good	7	32.9
4	D.P.	M	55	40	Good	10	37·5
5	C.F.	F	42	30	Poor	16	119·9
6	E.W.	F	46	20	Poor	11	131 1
7	S.D.	F	31	30	Poor	16	402·0
8	P.N.	F	33	30	Poor	6	108·0
9	R.B.	M	50	50	Poor	9	83·0
10	S.W.	F	38	40	Poor	20	148.0

approval of the local Health Board Ethical Sub-Committee. The therapeutic procedure was as described by Lakshmipathi *et al.* (1977) except that where an initial low intensity treatment regime failed to produce acceptable clinical improvement, the patient was transferred to the high intensity programme as used by Wolff *et al.* (1975) and by Melski *et al.* (1977). Patients showing total clearance with or without minimal residual activity, and those showing a rapidly progressive generalized improvement at the time of the study, were classed as 'good responders'; those who were showing only slow improvement, were stationary or worse, were classed as 'poor responders'. Table 1 summarizes the classification of clinical response and the treatments of the individual patients.

The 8-MOP obtained from Basotherm, GmbH. Biberach an der Riss, F.R.G., as Meladinine, 10 mg per tablet, was administered in a dosage of 20 mg for body weight 30–50 kg, 30 mg for 51–65 kg, 40 mg for 66–80 kg, and 50 mg for 81–90 kg.

Blood specimens (10 ml into lithium heparin tubes) were taken at time 0, before the oral administration of 8-MOP, and thereafter at 30 min, 1, 1½, 2, 3, 6, 12, and 24 h. The plasma was separated by centrifugation at 1,000 *g* for 10 min at room temperature and then stored at −20°C until assayed for 8-MOP.

Plasma 8-MOP analysis

Plasma samples (1·0 ml) were acidified with 0·5 ml *N*-HCL and after addition of sodium hexobarbitone as internal standard were extracted with 10 ml diethyl ether. After centrifugation, the ether layer was evaporated to dryness under nitrogen, re-dissolved in 100 μl of n-hexane:chloroform (60:40) and 5-20 μl injected for HPLC analysis.

HPLC absorption analysis was carried out using n-hexane: chloroform (60:40) as mobile phase, on a 5μ spherisorb S5W 12·5 cm, 4·6 mm internal diameter column. An Altex 110 HPLC pump was used with a flow rate of 3·0 ml/min with detection at 250 nm in a Pye Unicam LC3 detector. The system could detect 5 ng 8-MOP/ml plasma and the coefficient of variation at 10 ng/ml was 5·7%.

RESULTS

Plasma 8-MOP profiles showed similar patterns in the four 'good responders' and are shown as mean data ± s.e.mean in Fig. 1. There is a relatively rapid build up to a peak plasma concentration (123

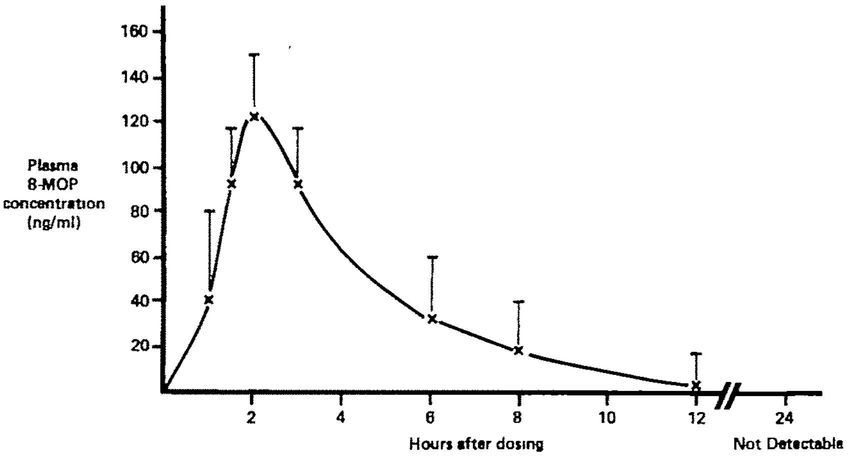

FIGURE 1. Plasma 8-MOP concentrations following a single oral dose in four patients showing a good response.

TABLE 2. Plasma 8-MOP pharmacokinetic parameters in good and poor responders

	Peak plasma 8-MOP concentration (ng/ml)	Time to peak plasma concentration (h)	Plasma 8-MOP elimination $t\frac{1}{2}$ (h)
Good responders (4)	125	2·0	1·90
Poor responders			
C.F. (female)	190	2·9	2·17
E.W. (female)	140	1·9	2·3
S.D. (female)	52	8·0	12·24
P.N. (female)	310	6 2	—
R.B. (male)	270	5·1	3·0
S.W. (female)	150	1·8	0 87

ng/ml) at 2 hr after ingestion followed by a similar rapid decay ($t\frac{1}{2}$ = 1·9 h) until little 8-MOP is detected in the plasma at 12 h with none detectable at 24 h. Plasma 8-MOP concentration versus time profiles for four of the six 'non-responders' patients were different and for all six subjects pharmacokinetic parameters are given in Table 2 along with, for comparison, the mean group data for the good responders. Two of the profiles, patients C.F. and E.W., are essentially similar to that for the group of good responders. That for patient S.D. shows a low peak value attained at 8 h post dosing, while patients P.N. and R.B., although reaching high peak plasma levels, did so at a significantly delayed time. In patient S.W. the 8-MOP concentration reached the normal range peak plasma concentration but fell abnormally rapidly thereafter.

DISCUSSION

In this study the plasma 8-MOP concentration versus time profiles obtained in subjects who responded well to the treatment is typical of the normal pharmacokinetic behaviour of the drug. It is similar to that reported by other authors (Steiner *et al.*, 1977; Thune & Volden, 1977; Busch *et al.*,

1978) and represents a relatively rapid absorption from the gut followed by an almost equally rapid clearance. Metabolic and isotope studies (Pathak *et al.*, 1974; Gazith *et al.*, 1978; Mandula & Pathak, 1978) have shown this clearance to result from rapid metabolism of the parent compound, the metabolites being eliminated mainly in the urine. The peak plasma levels obtained in the various studies are similar. However, Gazith *et al.* (1978) found the maximum to occur at an earlier time after ingestion, a difference attributed to drug preparation rather than variation in subject population. Thune (1978) reported similar results when using a new formulation of the drug and showed that the time of peak skin photosensitivity was also changed to correlate with that of peak plasma concentration at 1 h after ingestion.

Although there is no apparent correlation between peak plasma levels of 8-MOP and the efficacy of photochemotherapy (Steiner *et al.*, 1978; Wagner *et al.*, 1979), a variation from the normal pharmacokinetic behaviour might be expected to result in a change in the effect of the treatment. Similarly, where the treatment is shown to have little effect, an abnormal pharmacokinetic profile for the patient concerned might be expected. In this present study four of our poor responders showed abnormal plasma 8-MOP profiles. With the other two subjects studied no obvious abnormality occurred. Where the peak plasma concentration is delayed or premature, the time of exposure to UV-A might be adjusted accordingly. Where the peak level is lower than normal as well as delayed, the dose of 8-MOP administered might be increased as well. Wagner *et al.* (1979) showed in three patients that such adjustments to the routine photochemotherapy regime were indeed beneficial. It is apparent that where the routine of photochemotherapy with 8-MOP and UV-A is not effective, an examination of the pharmacokinetic behaviour of the drug in that patient may be of value and may show how the treatment should be adjusted to become effective. However, it is possible that the drug plasma profile in 'non-responders' may be completely normal and that the lack of response in these patients may be due more to some abnormality in drug penetration into the skin or in individual cellular responses to the phototoxic reaction involved or to other factors responsible for the psoriatic reaction.

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REFERENCES

Busch, U., Schmid, J., Koss, P.F., Zipp, H. & Zimmer, A. (1978) Pharmacokinetics and metobolite pattern of 8-methoxypsoralen in man following oral administration as compared to the pharmacokinetics in rat and dog. *Archives of Dermatological Research*, **262**, 255.

Gazith, J., Schalla, W. & Schaefer, H. (1978) 8-methoxypsoralen gas chromatographic determination and serum kinetics. *Archives of Dermatological Research*, **263**, 215.

Hensby, C.N. (1978) The qualitative and quantitative analysis of 8-methoxypsoralen by HLPC-UV and GLC-MS. *Clinical and Experimental Dermatology*, **3**, 355.

Herfst, M.J., Koot-Gronsveld, E.A.M. & De Wolff, F.A. (1978) Serum levels of 8-methoxypsoralen in psoriasis patients using a new fluorodensitometric method. *Archives of Dermatological Research*, **262**, 1.

Kligman, A.M. & Goldstein, F.D. (1973) Oral dosage of methoxsalen phototoxicity. *Archives of Dermatology*, **107**, 549.

Lakshmipathi, T., Gould, P.W., Mackenzie, L.A., Johnson, B.E. & Frain-Bell, W. (1977) Photochemotherapy in the treatment of psoriasis. *British Journal of Dermatology*, **96**, 587.

Mandula, B.B. & Pathak, M.A. (1978) Metabolic reactions *in vitro* of psoralens with liver and epidermis. *Biochemical Pharmacology*, **28**, 127.

Melski, J.W., Tanenbaum, L., Parrish, J.A., Fitzpatrick, T.B. & Bleich, H.L. (1977) Oral methoxsalen photochemotherapy for the treatment of psoriasis: a cooperative clinical trial. *Journal of Investigative Dermatology*, **68**, 328.

PATHAK, M.A., DALL'ACQUA, F., RODIGHIERO, G. & PARRISH, J.A. (1974) Metabolism of psoralens. *Journal of Investigative Dermatology*, **62**, 347.

STEINER, I., PREY, T., GSCHNAIT, F., WASHUTTL, J. & GREITER, F. (1977) Serum level profiles of 8-methoxypsoralen after oral administration. *Archives of Dermatological Research*, **259**, 299.

STEINER, I., PREY, T., GSCHNAIT, F., WASHUTTL, J. & GREITER, F. (1978) Serum levels of 8-methoxypsoralen 2 h after oral administration. *Acta Dermato-venerealogica*, **58**, 185.

SWANBECK, G., THYRESSON-HOK, M., BREDBERG, A. & LAMBERT, B. (1975) Treatment of psoriasis with oral psoralens and longwave ultraviolet light. *Acta Dermato-venerealogica*, **55**, 367.

THUNE, P. (1978) Plasma levels of 8-methoxypsoralen and phototoxicity studies during PUVA treatment of psoriasis with meladinin tablets. *Acta Dermato-venerealogica*, **58**, 149.

THUNE, P. & VOLDEN, G. (1977) Photochemotherapy of psoriasis with relevance to 8-methoxypsoralen plasma level at low intensity irradiation. *Acta Dermato-venerealogica*, **57**, 351.

WAGNER, G., HOFMANN, C., BUSCH, U., SCHMID, J. & PLEWIG, G. (1979) 8-MOP plasma levels in PUVA problem cases with psoriasis. *British Journal of Dermatology*, **101**, 285.

WOLFF, K., PARRISH, J.A., FITZPATRICK, T.B., GSCHNAIT, F., GILCHREST, B., HONIGSMANN, H. & PATHAK, M.A. (1975) Photochemotherapy of psoriasis with oral psoralens. *Journal of Investigative Dermatology*, **64**, 295.

WULF, H.C. & HART, J. (1979) Distribution of tritium-labelled 8-methoxypsoralen in the rat, studied by whole body autoradiography. *Acta Dermato-venerealogica*, **59**, 97.

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Dapsone in the treatment of lupus erythematosus

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SUMMARY

Dapsone yielded excellent therapeutic results in certain forms of lupus erythematosus (LE), whereas discoid lesions and the maculo-papular rash of the systemic and disseminated chronic forms of discoid LE remained uninfluenced by the drug. On the basis of these observations, we suggest the following indications for dapsone treatment in LE:

(1) Vasculitic urticaria.
(2) Oral ulceration.
(3) Non-scarring form of chronic LE.
(4) Chloroquine intolerance.

Besides its routine use in leprosy, dapsone has become standard therapy in dermatitis herpetiformis. Furthermore, good results have been reported in bullous dermatoses, pustular eruptions, different forms of vasculitis and acne conglobata (reviewed by Lang, 1979).

Matthews, Saihan & Warin (1978) reported a dramatic improvement of urticarial lesions in systemic lupus erythematosus (SLE) with dapsone. We observed a similar patient, and obtained excellent responses in two patients with the non-scarring form of chronic discoid LE (Bielicky, 1963) and a patient with severe oral ulceration associated with LE. Two patients with SLE and widespread cutaneous lesions and a patient with disseminated chronic LE were completely unresponsive to dapsone.

CASE REPORTS

Case 1

A 33-year-old woman suffering from SLE for 8 years (Raynaud's phenomenon, alopecia, leukopenia, positive ANA, dsDNA-antibody 64 iu/ml (normal <25), IgG deposits at the basement membrane of involved and uninvolved skin, marked lowering of CH50, C1, C2, C3 and C4). Repeated episodes of urticaria could be completely suppressed within 48 h by 100 mg dapsone daily. Prompt reappearance of the urticaria followed withdrawal of the drug. During the whole observation period the

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patient received 10 mg prednisolone and 100 mg azathioprine daily. Three months after institution of dapsone, CH50, C1 and C2 returned to normal values, whereas C3 remained slightly and C4 markedly depressed (Ruzicka & Goerz, in press).

Case 2

This 33-year-old male patient had a 9-year history of extensive oral ulceration recurring every 4–6 weeks, which responded to local corticosteroid therapy. The condition was assumed to be 'aphthosis'. In addition, infiltrated, bluish-red hyperkeratotic plaques were present on the cheeks and dorsa of the hands. They were not worsened by sun exposure. An extremely severe bout of oral ulcers resistant to standard therapy led eventually to hospitalization. On examination, extensive erosions and punched-out ulcers were seen on the soft and hard palate, lips, cheeks and tongue. ENT-examination revealed extension of the lesions onto the epiglottis and larynx. Furthermore, several urticarial lesions were noted. Parenteral feeding was instituted, as oral intake of even fluids was impossible. The cutaneous lesions raised the suspicion of lupus erythematosus, which was confirmed by histology from the dorsum of the hand. Direct immunofluorescence histology showed a lupus band at the basement membrane of the lesional skin with deposits of IgG and was negative in clinically healthy skin. No further ARA criteria (Cohen *et al.*, 1971) were fulfilled. LE-cells, ANA, dsDNA-antibodies and virus antibodies were negative, as well as routine laboratory examinations, except for an ESR of 40 mm/h. Discoid LE with oral ulceration was diagnosed. Therapy with 100 mg dapsone daily resulted in complete healing of the oral lesions within 2 weeks and disappearance of the urticaria within 3 days. The remission was maintained with dapsone alone for 2 months. As the cutaneous discoid lesions showed no change, chloroquine 250 mg daily was added. Within an observation period of 6 months, no further oral or urticarial lesions have appeared, and minimal scarring was seen as a sequel of the old ulcers.

Case 3

This 10-year-old Turkish girl developed, after a summer holiday in Turkey, a maculo-papular butterfly eruption on her cheeks. The rash persisted for 4 months. On admission, bright-red, rather ill-defined, exudative confluent papules were present over the bridge of the nose and cheeks forming by confluence the typical butterfly configuration. There was no atrophy or ulceration, and only slight follicular hyperkeratosis. Clinically, the non-scarring form of DLE was diagnosed. Histological examination was typical of chronic LE. Pertinent laboratory investigations yielded normal values with the exception of slight eosinophilia (650/μl). Dapsone, 25 mg daily, and 500 mg vitamin C daily resulted in almost total clearing of the lesions within 3 weeks. The remission has been maintained with this medication alone for up to 6 months.

Case 4

A 33-year-old male patient. Two years ago he noted a red spot on his forehead slowly increasing in size. One year ago DLE was diagnosed by his physician and 500 mg chloroquine daily was prescribed. After 2 weeks an allergic maculo-papular rash appeared necessitating discontinuation of the treatment. He remained without therapy for 1 year. After intensive sun exposure an exacerbation occurred and he was referred to our department. On admission, red, ill-defined, slightly raised papules with telangiectasia were noted on the forehead, cheeks, nose and neck. Histological examination showed LE. Complete laboratory and clinical investigations gave normal findings. Dapsone, 100 mg daily, led to a partial but unequivocal response within 14 days. Complete clearing of the lesions was achieved with the addition of 8 mg methyl-prednisolone daily. No relapse occurred during the following 4 months while he was maintained on both drugs.

Case 5

A 34-year-old female patient suffering for 3 years from SLE with widespread cutaneous lesions (latex agglutination test for rheumatoid factor and Waaler-Rose test positive, ANA positive, alopecia, sensitivity to sun exposure, C3 and C4 within normal range, ds DNA-antibodies negative). Up to 150 mg dapsone daily had no effect on the cutaneous lesions.

Case 6

A 25-year-old female patient with SLE for 5 years (LE-cells, ANA, rheumatoid factors—latex agglutination and Waaler-Rose test—positive, leukopenia, occasional oral lesions, sensitivity to sun. dsDNA-antibodies negative). Up to 150 mg dapsone daily did not influence the widespread maculopapular skin lesions.

Case 7

A 44-year-old female patient. Six months ago she noted profuse loss of hair and widespread livid-red macules mainly on her face, neck, extensor aspects of her arms and dorsa of the hands. On examination, typical macular or slightly elevated lesions of disseminated chronic LE were noted; the diagnosis was confirmed by histology and immunofluorescence. Clinical and laboratory signs of systemic involvement were absent apart from alopecia. No therapeutic effect was observed with up to 150 mg dapsone daily for 3 weeks. Chloroquine, 250 mg daily, resulted in marked improvement of the dermatosis.

COMMENT

Despite its wide use in dermatology the mechanism of action of dapsone is unknown. It is believed to influence the action of neutrophils since an effect is observed in diseases characterized by neutrophilic infiltration (Person & Rogers, 1977). Its influence on lysosomes is contested (Barranco, 1974; Mier & Van den Hurk, 1975).

Urticarial lesions with underlying vasculitis seem to be quite common in SLE (O'Loughlin, Schroeter & Jordon, 1978). Matthews *et al.* (1978) observed a rapid clearing of these lesions with dapsone in a patient with this disease who exhibited low serum levels of CH50, C1,2,3 and C1q. A similar observation was made recently by Highet (1980). We reproduced this impressive therapeutic effect in a patient with SLE, urticaria and intensive activation of the classical complement pathway. Thus, we are able to confirm the efficacy of dapsone in vasculitic urticaria. (We observed a similarly rapid response to dapsone in a patient with widespread febrile neutrophil dermatosis of Sweet, often considered to be another disease with an underlying leukocytoclastic vasculitis—unpublished observation.)

Our second patient also exhibited urticaria responding to dapsone but the complete healing of massive oral ulceration is of interest. Oral ulceration is common in SLE but less common in DLE. Our patient did not fulfil the ARA criteria for SLE and pertinent laboratory tests were negative. However, urticarial lesions and oral ulcers might be suggestive of the presence of circulating immune complexes. Dapsone seems worth while trying in mucous membrane lesions in LE. (Two patients with Behçet's disease showed no response to dapsone, one patient showed massive exacerbation of aphthous lesions—unpublished observations.) Finally, one patient with the non-scarring form of chronic LE as described by Bielicky (1963) showed a complete clearing of her fresh, exudative lesions, and another patient with this form of LE improved considerably with dapsone alone, but additional medication with glucocorticoids was required for complete control.

Thus, we suggest the following indications for dapsone treatment in lupus erythematosus:

(1) Vasculitic urticaria.
(2) Oral ulceration.
(3) Non-scarring form of chronic discoid LE.
(4) Chloroquine intolerance.

REFERENCES

BARRANCO, V.P. (1974) Inhibition of lysosomal enzymes by dapsone. *Archives of Dermatology*, **110**, 563.

BIELICKY, T. (1963) Electrophoretic plasma pattern and erythrocyte sedimentation in scarring and non-scarring forms of chronic lupus erythematosus. *Acta Dermato-Venereologica*, **43**, 293.

COHEN, A.S., REYNOLDS, W.E., FRANKLIN, E.C. *et al.* (1971) Preliminary criteria for the classification of systemic lupus erythematosus. *Bulletin on the Rheumatic Diseases*, **21**, 643.

HIGHET, A.S. (1980) Urticarial vasculitis resembling systemic lupus erythematosus: efficacy of prednisone and dapsone combined. *British Journal of Dermatology*, **102**, 358.

LANG, P.G. (1979) Sulfones and sulfonamides in dermatology today. *Journal of the American Academy of Dermatology*, **1**, 479.

MATTHEWS, C.N.A., SAIHAN, E.M. & WARIN, R.P. (1978) Urticaria-like lesions associated with systemic lupus erythematosus: response to dapsone. *British Journal of Dermatology*, **99**, 555.

MIER, P.D. & VAN DEN HURK, J.J.M.A. (1975) Inhibition of lysosomal enzymes by dapsone. *British Journal of Dermatology*, **93**, 471.

O'LOUGHLIN, S., SCHROETER, A.L. & JORDON, R.E. (1978) A study of lupus erythematosus with particular reference to generalized discoid lupus. *British Journal of Dermatology*, **99**, 1.

PERSON, J.R. & ROGERS, R.S. (1977) Bullous and cicatricial pemphigoid. Clinical, histopathologic and immunopathologic correlations. *Mayo Clinic Proceedings*, **52**, 54.

RUZICKA, T. & GOERZ, G. Systemic lupus erythematosus and vasculitic urticaria: effect of dapsone and complement levels. *Dermatologica*, in press.

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Pharmacology and Treatment

The Duhring chamber assay for corticosteroid atrophy

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SUMMARY

A new human model for the evaluation of the atrophogenicity of topical corticosteroids is proposed. Formulated corticosteroids (creams and ointments) were applied occlusively to normal forearm skin of human volunteers for 3 weeks using Duhring chambers. Visible atrophy and telangiectasia were rated under a stereomicroscope on a histologically validated five-point scale.

There was a close correlation between the parameters of atrophy and telangiectasia. Atrophogenicity varied considerably among the tested corticosteroids, ranging from none (1% hydrocortisone) via moderate (0·1% betamethasone valerate) to very severe (0·05% clobetasol propionate). A dose-response relationship with exposure time and concentration of the corticosteroid could be demonstrated. Furthermore, the vehicle influenced the resulting atrophy and telangiectasia. Fatty ointments seem to pose less risk than oil in water creams.

Reviewing the present status of experimental models to assess the atrophogenicity of corticosteroids, Dykes & Marks (1979) concluded that we are still far from the ideal technique. Due to the uniqueness of human skin with its well developed papillary dermis and microvasculature, data from animal experiments are difficult to interpret in predicting the atrophy risk for human skin. The ideal human model should be reliable, practical and non-invasive, i.e. non-scarring and without any other hazards such as radiation (Black, 1969). Furthermore, a simultaneous comparison of several products on one subject is desirable for statistical reasons.

We have developed such a technique. The corticosteroid preparations are applied to normal forearm skin under occlusion for three weeks. Resulting atrophy and telangiectasia are evaluated according to visible changes seen with a stereomicroscope. By use of special aluminium chambers for accurate drug exposure up to twelve products can be compared to each other in one test.

MATERIALS

All test materials except hydrocortisone were marketed corticosteroid preparations, which were packed into neutral tubes and coded until completion of the study.

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Creams; ointments

Difluocortolone valerate 0·1% water/oil cream (DFV); hydrocortisone acetate 1·0% in DFV w/o cream base; betamethasone 17-valerate 0·1% oil/watercream (BMV), betamethasone 17-21-dipropionate 0·05% o/w cream (BDP); hydrocortisone 17-butyrate 0·1% o/w cream (HCB); fluocinolone acetonide 0·025% and 0·2% o/w cream (FAC); triamcinolone acetonide 0·1% and 0·5% o/w cream (TAC); clobetasol 17-propionate 0·05% o/w cream (CBP); halcinonide 0·1% fatty alcohol-propylene glycol (FAPG) ointment; fluocinonide 0·05% FAPG ointment (FLU).

Fatty ointments

DFV 0·1%, BDP 0·05%, FLU 0·05% and HCB 0·1%.

The steroid-free cream and ointment bases of HAL and DFV were supplied by the hospital dispensary. The bases of FLU and HCB (cream and ointment) were supplied by the manufacturer.

PROCEDURE

The test materials were applied to the ventral aspect of normal forearm skin of human volunteers by use of Duhring chambers. These aluminium chambers with an elevated margin ensure perfect occlusive exposure of a volume of 0·1 ml to a circular area of 12 mm diameter and have proven to be superior to the conventional patch test (Frosch & Kligman, 1979). Up to six chambers can be fixed to the mid-forearm by encircling roles of porous tape (Micropore, 3 M Co.). The test materials were renewed three times weekly for a total exposure period of 3 weeks. One day after stopping the application, the exposed skin was examined under a stereomicroscope (Operationsmikroskop Op Mi 1, Zeiss, Oberkochen, magnification 0·6–12×). Two parameters, atrophy and telangiectasia, were rated on a five-point scale on the following basis.

Atrophy

The thickness of the superficial skin, its dermatoglyphic pattern, and any increase in transparency for the underlying vasculature were assessed as follows: 0—no change; 1+—slight transparency increase and slight irregularity of the dermatoglyphic pattern; 2+—moderate thinning of the epidermis with moderate increase in transparency and flattening of furrows and ridges; 3+—severe thinning and increase in transparency, the dermatoglyphic pattern being severely effaced; 4+—very severe thinning of the epidermis with complete loss of dermatoglyphics ('glazed skin'), the vasculature appearing to be directly under the surface.

Telangiectasia

The visibility of the vasculature was greatly enhanced by a drop of mineral oil under a glass slide, particularly for photographic purposes. Assessments were made as follows: 0—normal vascular pattern with fine capillary loops under highest magnification; 1+—capillary hyperaemia with slight elongation and dilatation of blood vessels not visible to the naked eye; 2+—moderate telangiectasia, just visible with the naked eye; 3+—severe telangiectasia with marked reduction of capillary loops; 4+—very severe telangiectasia with large blunt vessels and absence of capillary loops.

An example of extreme atrophy and telangiectasia, rated both as 4+, is illustrated in Fig. 1.

Readings were taken independently by at least two investigators. The arithmetical mean of both was taken as the final score. (Reproducibility of the subjective readings could be improved by a 'learning period' including serial photographs until good agreement was achieved.) Deviations of individual scores never exceeded one point and the total error was less than 10%. Telangiectasia

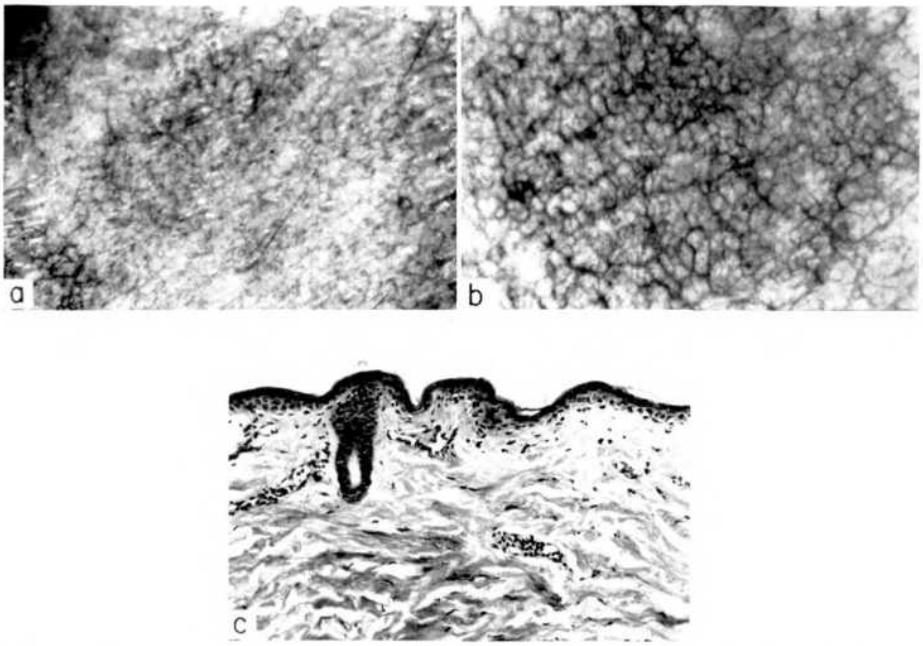

FIGURE 1. Severe atrophy, rated 4+ under the stereomicroscope (a). Visibility of telangiectasia (4+) is improved by viewing under oil and a cover slip (b). Histological examination (c) confirms extreme epidermal atrophy, effacement of the papillary dermis and marked vascular dilatation. (Magnification: a, b × 17·5; c × 250).

could be judged more easily than atrophy and scores obtained by the investigators tended to be closer. In addition photodocumentation of telangiectasia was of better quality than for epidermal thinning and increase in transparency.

HISTOLOGICAL STUDIES

For validation of the above scoring system histological examination of treatment sites, rated differently for atrophy and telangiectasia, was performed. A total of twelve 4 mm punch biopsies were taken in five subjects using local anaesthesia. Routine histological processing and H & E staining were used.

EXPERIMENTS AND SUBJECTS

Healthy white volunteers of either sex, aged 18–35 years, with normal forearm skin were used. Informed written consent was obtained. Subject panels varied from 5 to 20 and are indicated below.

Study I

Twenty subjects were tested with the following series of twelve randomly applied corticosteroid creams and bases: HCA, DFV, BMV, BDP, FAC, TAC, HAL, CBP. BMV and CBP were included as duplicates. The steroid-free bases of HAL (BH) and DFV (BD) served as controls.

Study II

Ten subjects were tested with the active cream and fatty ointment preparations of DFV, HCB and FLU as well as with the corresponding steroid-free bases, amounting to a total of twelve test products.

Study III

For studying the time-response and dose-response relationships five subjects were tested as follows: 0·1% TAC, 0·5% TAC, 0·025% FAC and 0·2% FAC were applied for 3 weeks. DFV, BMV and CBP as a set of three test products of different atrophogenicity (see below) were exposed for 1 week, 2 weeks and 3 weeks. Readings were taken 1 day after drug discontinuance.

RESULTS

Histological studies

Skin considered to be highly atrophic by stereomicroscopy (3+ and 4+) always showed the histological criteria of atrophy: thinning of the stratum corneum and epidermis with effacement and marked reduction of the papillary dermis leading to a flat dermo-epidermal junction (Fig. 1c). The vascular changes paralleled those in the epidermis and dermis. Severely atrophic skin always showed marked dilatation of the capillaries and of the superficial venular plexus. The vessels appeared close to the epidermis due to wasting of the papillary dermis. Histological changes were less pronounced with decreased ratings for atrophy as well as for telangiectasia. Scores of 1+ revealed essentially normal skin upon microscopic investigation.

In summary, these observations showed that skin rated under the stereomicroscope to be atrophic and telangiectatic would indeed meet these criteria on histological examination. While scores of 1+ for both parameters may be due to optical effects or functional changes in the vasculature, scores of 2+ and higher are certainly meaningful and indicate atrophy of the epidermis and superficial dermis as well as profound vascular damage caused by corticosteroids.

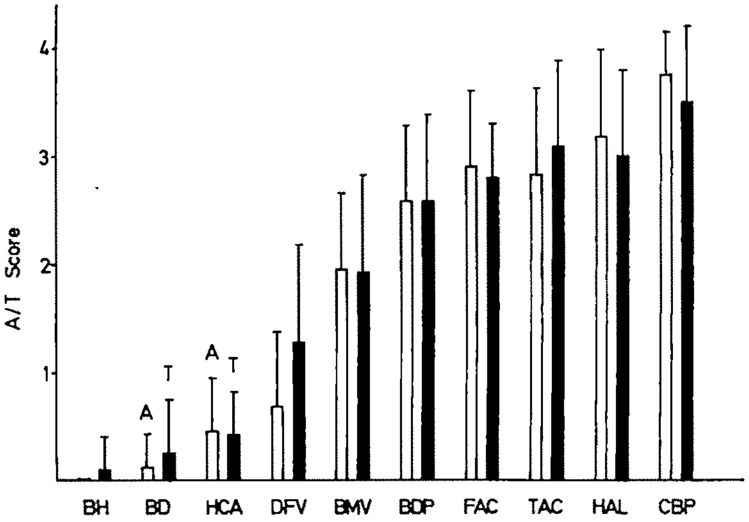

FIGURE 2. Study I. Atrophy (A) and telangiectasia (T) after 3 weeks of occlusive treatment with various corticosteroid creams and two bases (BH, BD). Mean score of twenty subjects (±s.d.).

Study I

Mean scores for atrophy and telangiectasia of twenty subjects tested with various corticosteroid creams for 3 weeks are illustrated in Fig. 2.

Both bases and HCA failed to produce any significant atrophy or telangiectasia (mean scores below 0·5). All other steroids produced more atrophy and telangiectasia ranging from slight to very severe in the following ascending order: DFV, BMV, BDP, FAC, TAC, HAL, CBP. Analysis of variance with Tukey test for differences revealed statistical significance for atrophy scores ⩾0·6 and for telangiectasia scores ⩾0·5 ($P<0{\cdot}05$). The two materials studied in duplicate, BMV and CBP, produced the same amount of atrophy and telangiectasia with the largest deviation of 0·17 in mean scores, a statistically insignificant difference. There was an excellent correlation between scores for atrophy and telangiectasia ($r = 0{\cdot}91$, $P<0{\cdot}01$). Hence, for the following studies only the parameter of telangiectasia was considered.

TABLE 1. Study II. Mean telangiectasia scores (±s.d.) of ten subjects treated with various corticosteroid creams, ointments and respective bases for 3 weeks under occlusion

	Cream	Ointment
DFV 0·1%	1·33±0·88	0·53±0.81
Base	0·20±0·32	0·23±0·42
FLU 0·05%	3·10±0·71	1·37±0·97
Base	0·77±0.68	0·47±0·55
HCB 0·10%	3·20±0·60	1·60±0·80
Base	0·90±0·60	0·60±0·50

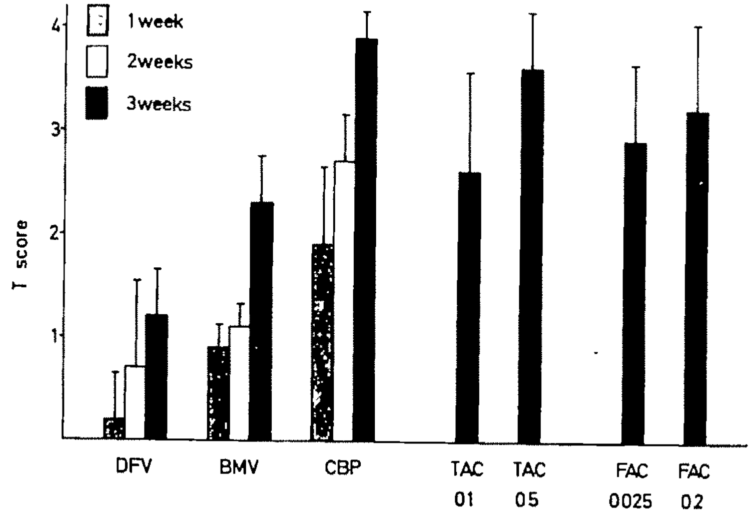

FIGURE 3. Study III. Telangiectasia after 1, 2 and 3 weeks of occlusive treatment with DFV, BMV and CBP. TAC and FAC were applied for 3 weeks at two different concentrations.

Study II

The results of this study on the influence of the vehicle upon resulting telangiectasia are given in the table. Statistical analysis by the same method as used for study I revealed significance for differences in telangiectasia $\geqslant 0{\cdot}7$ ($P < 0{\cdot}05$). On this basis all corticosteroid creams produced more telangiectasia than their bases. When the same corticosteroid was applied in a fatty ointment the resulting telangiectasia was markedly less developed. For 0·1% HCB and 0·05% FLU there was still a significant difference to the ointment bases. 0·1% DFV ranked lowest in both vehicles compared to the other two steroids.

Study III

The dose-response relationships are illustrated in Fig. 3. The intensity of telangiectasia increased with the exposure time from 1 week to 3 weeks and was most clearly demonstrated by CBP. Using different strengths of the same corticosteroid, 0·5% TAC produced more telangiectasia than 0·1 TAC while the difference was less obvious for FAC 0·025% and FAC 0·2%.

DISCUSSION

This is a simple, non-invasive technique where histologically proven corticosteroid atrophy and telangiectasia are recognized under a stereomicroscope *in vivo* on human volunteers. Semiquantitative assessment of the atrophogenicity is based on scoring the visual changes regarding skin surface structure, transparency and telangiectasia on a five-point scale. Thus, up to 12 formulated corticosteroid creams or ointments can be ranked according to the severity of their skin-thinning effects within 3 weeks on a panel of ten to twenty subjects.

A major advantage, compared with other models for assessment of corticosteroid atrophy, is that in this assay epidermal and dermal effects are evaluated at the same time *in vivo*. Based on our histological studies, the appearance of atrophy and transparency of skin is due to thinning of the epidermis and stratum corneum as well as to effacement and shrinkage of the papillary dermis. These changes are accompanied by dilatation and elongation of the fine capillary loops in the dermal papillae and approximation of the highly dilated superficial venular plexus to the flat dermo-epidermal junction. These vascular changes are visible clinically as telangiectasia.

Severe (3+ and 4+) atrophy and telangiectasia required 4–8 weeks for complete disappearance as a follow up of study I has shown. Visible atrophy and telangiectasia can therefore not be explained by optical or functional effects, e.g. reflectory vasodilation after prolonged vasoconstriction or compression by the chamber.

While Montagna (1956) held that the opacity of normal skin was due to the constitution of the epidermis we are in agreement with McConkey *et al.* (1967) that transparency of the skin requires changes in the dermal collagen and ground substance. Their histological studies on patients suffering from rheumatoid disease and osteoporosis revealed that transparent appearing skin frequently had a thinner and less keratinized epidermis than opaque skin. A more constant feature, however, was thinning of the collagen fibres and increase of the interstitial ground substance, being most marked in the upper third of the dermis. The collagen content of transparent skin turned out to be only half that of normal skin. McConkey *et al.* (1967) explained these findings by assuming a specific defect in collagen maturation since collagen biosynthesis was not found to be decreased in comparison to opaque skin. These investigators also observed that transparency of the skin became more marked with prolonged (systemic) corticosteroid therapy.

A clinical hallmark of long-term topical corticosteroid application is telangiectasia (Sneddon, 1969).

According to Champion (1968) telangiectasia is found in most processes that cause atrophy of the skin. Several investigators have described an association of cutaneous atrophy with telangiectasia after corticosteroid injection (Schetman, Hambrick & Wilson, 1963; Stankler & Ewen, 1972; Snyder & Greenberg, 1977) as well as after prolonged topical application (Stevanovic, 1972; Smith, Wehr & Chalker, 1976).

Stankler & Ewen (1972) inspected the skin with the unaided eye at a distance of 1 foot and noted only whether telangiectasia was present or absent. Histological examination of telangiectatic skin revealed marked epidermal and dermal atrophy in all cases. However, slight atrophic changes on a histological basis were not always associated with clinical telangiectasia. This might well be explained by the insensitive technique of detecting telangiectasia with the naked eye since Stankler & Ewen (1972) did find 'telangiectasia' upon histological examination.

Snyder & Greenberg (1974) used a dissecting microscope and found telangiectasia with decreased number of capillary loops in severe dermal atrophy after a palpable depression due to corticosteroid injection had developed.

As we found a good correlation between epidermal atrophy, atrophy of the papillary dermis and severity of telangiectasia both at a histological and 'clinical' level under the stereomicroscope we suggest that the parameter telangiectasia is a sensitive indicator of steroid atrophy. We are aware of a possible dissociation between epidermal and dermal effects of a corticosteroid although no convincing data for this have been presented so far in our view.

Both the sensitivity and the reproducibility of the Duhring chamber assay are good although a subjective scoring system is used. This may be concluded from the duplicates tested in study I and the comparison of values for DFV, BMV and CBP in studies II and III respectively. The response depended on the application time and concentration of the corticosteroid. While 0·5% TAC was clearly more atrophogenic than 0·1%, the smaller difference for an eight-fold increase of FAC from 0·025% to 0·2% suggests that the two corticosteroids varied as to the effects of concentration on the resultant atrophy.

Comparing the atrophic potential of individual corticosteroids based on this assay it must be emphasized that the relative rank order rather than the absolute scores are meaningful. Mean scores for steroid-free bases may vary and ranged from 0 to 0·9 with the bases used in these studies. Therefore it is preferable to include the bases if corticosteroids in different bases are to be evaluated.

Study II revealed an important influence of the vehicle on resulting telangiectasia (and atrophy). Corticosteroid fatty ointments turned out to be less atrophogenic than creams. A non-fluorinated corticosteroid, HCB, induced severe side effects both as cream and ointment. These differences might be explained as follows. Firstly, the release of the steroid depends very much on the vehicle and may be markedly less from a fatty ointment than from an oil in water cream. Release from either vehicle could be greatly enhanced by addition of propylene glycol (Polano & Ponec, 1976). Secondly, some cream bases are irritating after prolonged exposure, particularly under occlusion (manuscript in preparation). This may not become evident due to the anti-inflammatory action of the corticosteroid in the active preparation. Irritated skin has a higher potential to develop corticosteroid atrophy than completely normal skin, as Stankler & Ewen (1972), Snyder & Greenberg (1974) and our own group (Frosch & Behrenbeck, 1980) have demonstrated. Irritation from the vehicle may thus enhance the resulting atrophy in this test since occlusion over a period of 3 weeks is used. This may lead to overrating of some corticosteroid creams. Irritation can always be differentiated from atrophy and telangiectasia due to corticosteroids as examination of test sites treated with various primary irritants has shown (sodium lauryl sulphate, croton oil, propylene glycol). If the same corticosteroid still produces significant atrophy and telangiectasia in a fatty ointment base, as HCB and FLU have in study II, the atrophogenic potential of these materials must be considered to be substantial.

Finally, our results have to be compared with those of other reported techniques for assessment of corticosteroid atrophy. In humans atrophy due to hydrocortisone is rare even after application for months and remains confined to few exotic cases reported by Mancini, Stringa & Canepa (1960) and Stevanovic (1972).

Using the radiographic technique Dykes & Marks (1977) found the greatest thinning of the skin after twice daily application over 4 weeks with CBP (17%), followed closely by HCB (13%). Surprisingly 0·1% BMV was just as effective as 1% hydrocortisone in thinning of the skin (6% and 7% respectively). With the same method Snyder & Greenberg (1977) described marked atrophy with 0·1% TAC cream after 8 weeks of thrice daily application, while 1% hydrocortisone did not differ from the base. James, Black & Sparkes (1977) also used the X-ray technique and found significant changes with the following creams which were applied twice daily without occlusion for 3 months: HCB, FAC, flurandrenolone and clobetasone butyrate. This is in full agreement with our results including flurandrenolone after 3 weeks of occlusive treatment (Frosch & Behrenbeck, 1980).

Since corticosteroids have many facets of action evidence is mounting that there is a dissociation between anti-inflammatory and atrophogenic effects for some corticosteroids. Stevanovic, Wilson & Sparkes (1977) found clobetasone butyrate clinically to be more effective in psoriasis and eczema than flurandrenolone ointment yet to cause less epidermal thinning. DFV may be another example of a favourable risk/benefit ratio. In a large clinical trial on various dermatoses it could be demonstrated that 0·1% DFV as oily cream and fatty ointment was comparable or superior to the corresponding formulations of 0·1% BMV, 0·05% FLU and 0·025% FAC (Reckers & Wendt, 1976). Based on the results of the Duhring chamber assay DFV is a corticosteroid with a relatively low atrophogenicity in comparison to its clinical efficacy. This assay may be helpful in identifying new compounds with low cutaneous adverse effects such as visible thinning of the skin with telangiectasia.

TRADE NAMES AND MANUFACTURERS

Difluocortolone valerate—Nerisona Salbe, Nerisona Fettsalbe (Schering AG). Betamethasone valerate—Betnesol-V-Creme, Betnesol-V-Salbe (Glaxo). Betamethasone dipropionate—Diprosone Creme, Diprosone Salbe (Byk-Essex). Fluocinolone acetonide—Jellin Creme 0·025% and Jellin-ultra-Creme 0·2% (Grünenthal). Fluocinonide—Topsym Salbe, Topsym Fettsalbe (Grünenthal). Halcinonide—Halog Salbe (Heyden). Triamcinolone acetonide—Volon A Creme 0·1% (Heyden), Kenalog Cream 0·5% (Squibb). Clobetasol propionate—Dermoxin Creme (Glaxo). Hydrocortisone butyrate—Alfason Creme, Alfason Salbe (Thomae). Steroid-free bases—Halog Basiscreme (Heyden), Neribas Salbe, Neribas Fettsalbe (Schering AG).

REFERENCES

BLACK, M.M. (1969) A modified radiographic method for measuring skin thickness. *British Journal of Dermatology*, **81**, 661.

CHAMPION, R.H. (1968) Telangiectases. In: *Textbook of Dermatology*, (Ed by A. Rook, D.S. Wilkinson and F.J. Ebling), p. 407. Blackwell Scientific Publications, Oxford.

DYKES, P.J. & MARKS, R. (1977) Measurement of skin thickness: a comparison of two *in vivo* techniques with a conventional histometric method. *Journal of Investigative Dermatology*, **69**, 275.

DYKES, P.J. & MARKS, R. (1979) An appraisal of the methods used in the assessment of atrophy from topical corticosteroids. *British Journal of Dermatology*, **101**, 599.

FROSCH, P.J. & KLIGMAN, A.M. (1979) The Duhring chamber. An improved technique for epicutaneous testing of irritant and allergic reactions. *Contact Dermatitis*, **5**, 73.

FROSCH, P.J. & BEHRENBECK, E.M. (1980) Hautatrophie durch verschiedene Corticosteroide im Ammoniumhydroxid-Blasen-Test sowie im Duhring-Kammer-Test am Menschen. *Aktuelle Dermatologie*, **6** (Suppl. 1) 51.

James, M.P., Black, M.M. & Sparkes, C.G. (1977) Measurement of dermal atrophy induced by topical steroids using a radiographic technique. *British Journal of Dermatology*, **96**, 303.

Mancini, R.E., Stringa, S.G. & Canepa, L. (1960) The action of ACTH, cortisone and prednisone on the connective tissue of normal and sclerodermic human skin. *Journal of Investigative Dermatology*, **34**, 393.

McConkey, B., Walton, K.W., Carney, S.A., Lawrence, J.C. & Ricketts, C.R. (1967) Significance of the occurrence of transparent skin. *Annals of the Rheumatic Diseases*, **26**, 219.

Montagna, W. (1956) *The Structure and Function of the Skin.* p. 15. Ser. 51, Academic Press, New York.

Polano, M.K. & Ponec, M. (1976) Dependence of corticosteroid penetration on the vehicle. *Archives of Dermatology*, **112**, 675.

Reckers, R. & Wendt, H. (1976) Multizentrische klinische Prufung des neuen Corticosteroids Difluocortolon valerianat als Creme, Salbe und Fettsalbe. *Drug Research*, **26**, 1499.

Schetman, D., Hambrick, G.W. & Wilson, C.E. (1963) Cutaneous changes following local injection of triamcinolone. *Archives of Dermatology*, **88**, 820.

Smith, J.G., Wehr, R.F. & Chalker, D.K. (1976) Corticosteroid-induced cutaneous atrophy and telangiectasia. *Archives of Dermatology*, **112**, 115.

Sneddon, I.B. (1969) Adverse effects of topical fluorinated corticosteroids in rosacea. *British Medical Journal*, **i**, 671.

Snyder, D.S. & Greenberg, R.A. (1974) Evaluation of atrophy production and vasoconstrictor potency in humans following intradermally injected corticosteroids. *Journal of Investigative Dermatology*, **63**, 461.

Snyder, D.S. & Greenberg, R.A. (1977) Radiographic measurement of topical corticosteroid-induced atrophy. *Journal of Investigative Dermatology*, **69**, 279.

Stankler, L. & Ewen, S.W.B. (1972) The effects of corticosteroid injections at sites of skin damage. *Journal of Investigative Dermatology*, **59**, 394.

Stevanovic, D.V. (1972) Corticosteroid-induced atrophy of the skin with telangiectasia. *British Journal of Dermatology*, **87**, 548.

Stevanovic, D.V., Wilson, L. & Sparkes, C.G. (1977) A separation of clinical from epidermal thinning effect in the topical glucocorticoid clobetasone butyrate. *British Journal of Dermatology*, **96**, 67.

British Journal of Dermatology (1981) **104**, 67.

Colchicine in the treatment of the cutaneous manifestations of Behçet's disease

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SUMMARY

Five patients with Behçet's disease were satisfactorily treated with colchicine. Oral aphthosis, erythema nodosum-like lesions and genital erosions improved greatly within a month as did laboratory findings. We believe colchicine to be the first choice in the management of the cutaneous and ocular lesions of Behçet's disease.

The enhanced chemotactic activity of polymorphonuclear leukocytes (PMNs) in patients with Behçet's disease was reported by Matsumura & Mizushima (1975) and PMNs may play a part in the pathogenesis of this disorder (Sobel *et al.*, 1977; Haim, 1979). In Japan, Behçet's disease is unusually common, and colchicine, a strong inhibitor of PMN chemotaxis, is sometimes used in the treatment of ocular attacks (Mizushima *et al.*, 1977) and by dermatologists for skin problems (Miyazawa, Ishibashi & Wada, 1978).

We here describe patients who improved greatly when treated with colchicine.

REPORT OF CASES

Case histories of the five middle-aged patients are summarized in Table 1. They had cutaneous manifestations such as erythema nodosum-like lesions, aphthosis, genital erosions and folliculitis-like lesions with or without ocular symptoms. During the last 1–8 years they had been treated with non-steroidal anti-inflammatory agents and/or corticosteroids with unsatisfactory results. Then they started on a regimen of 1 mg of colchicine a day by mouth and showed remarkable improvements of cutaneous as well as ocular manifestations within less than a month. Laboratory findings including elevated ESR and positive C-reactive protein (CRP) became within normal limits after the treatment. Our five patients have been doing well and have tolerated normal daily activities without recurrence of their symptoms or side effects while under treatment with 1 mg/day of colchicine for between 2 months and 2 years.

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TABLE 1. Summary of five cases

Case	1	2	3	4	5
Age/Sex	45/M	40/M	59/F	50/F	33/M
Symptoms					
Erythema nodosum-like lesions	+	−	+	+	+
Aphthosis	+	+	+	+	+
Genital erosions	−	+	−	−	+
Folliculitis-like lesions	−	+	−	−	−
Ocular symptoms	+	+	+	−	+
Period after the onset (years)	5	1	8	6	3
Previous treatments					
Indomethacin	+	−	+	−	−
Tiaramide	+	−	−	−	−
Potassium iodide	+	−	+	+	−
Naproxen	−	−	+	−	−
Corticosteroid	+	+	−	−	+
ESR (mm/h) before/after treatment	44/5	57/8	14/N.D.	64/N.D.	73/17
CRP before/after treatment	+/−	++/−	+/N.D.	++/N.D.	++/−
Follow-up period (months)	24	10	2	2	10

N.D.—Not determined.

DISCUSSION

Colchicine is an unique anti-inflammatory agent which has long been used in gouty arthritis, providing dramatic relief of acute attacks of gout. Though colchicine affects various leukocyte functions *in vitro*, the only leukocytic activity affected by the low colchicine plasma levels is known to be the inhibition of PMN chemotaxis (Wallace, Omokoku & Ertel, 1970).

As described above, the hyperreactive cellular inflammatory response characterizing Behçet's disease might be due to increased chemotactic activity of PMNs. Hence colchicine is a reasonable agent for the treatment of Behçet's disease. In the management of the mucocutaneous manifestations such as numerous small pustules, painful nodose lesions resembling erythema nodosum, oral aphthosis and genital erosions, non-steroidal anti-inflammatory drugs with or without systemic corticosteroids are frequently used by dermatologists. All patients in our series had been treated with non-steroidal anti-inflammatory drugs such as indomethacin, tiaramide and naproxen. However, skin lesions continued to appear. Corticosteroids are often effective in these skin manifestations, but they are not always of benefit for the ocular disorders (Urayama *et al.*, 1974). We have discontinued using corticosteroids due to the adverse systemic effects. Although potassium iodide, an inhibitor of PMN chemotaxis, is known to be effective for erythema nodosum and Sweet's syndrome (Horio *et al.*, 1980), it had no significant effect on Behçet's disease. This difference may provide clues for exploring the role of PMN's in the pathogenesis of Behçet's disease. Our five patients had been treated in vain with anti-inflammatory agents or we had been forced to abandon corticosteroids, so we were very satisfied with the results obtained with colchicine. It is of interest that colchicine has also been shown to be useful in the management of necrotizing vasculitis (Hazen & Michel, 1979) and familial Mediterranean fever (Dinarello *et al.*, 1976), a condition which bears some resemblance to

Behçet's disease (Urayama *et al.*, 1974). Though Mizushima *et al.* (1977) reported several side effects such as nausea, diarrhoea, oligospermia, amenorrhoea, general malaise and hair loss in one to eleven patients of their 131 cases, no undesirable effects of the drug were noticed during the courses of the treatment in our patients. However, the indications for treatment and the adverse effects will be carefully watched.

Colchicine may be considered the treatment of choice in the management of the skin manifestations of Behçet's disease.

REFERENCES

Dinarello, C.A., Chusid, M.J., Fauci, A.S. & Gallin, J.I. (1976) Effect of prophylactic colchicine therapy on leukocyte function in patients with familial Mediterranean fever. *Arthritis and Rheumatism*, **19**, 618.

Haim, S. (1979) The pathogenesis of lesions in Behçet's disease. *Dermatologica*, **158**, 31.

Hazen, P.G. & Michel, B. (1979) Management of necrotizing vasculitis with colchicine. *Archives of Dermatology*, **115**, 1303.

Horio, T., Imamura, S., Danno, K., Furukawa, F. & Ofuji, S. (1980) Treatment of acute febrile neutrophilic dermatosis (Sweet's syndrome) with potassium iodide. *Dermatologica*, **160**, 341

Matsumura, N. & Mizushima, Y. (1975) Leukocyte movement and colchicine treatment in Behçet's disease. *Lancet*, **2**, 813.

Miyazawa, T., Ishibashi, M. & Wada, K. (1978) Colchicine in the treatment of Beçhet's disease. *Rinsho derma*, **20**, 179.

Mizushima, Y., Matsumura, N., Mori, M., Shimizu, T., Fukushima, B., Mimura, Y., Saito, K. & Sugiura, S. (1977) Colchicine in Beçhet's disease. *Lancet*, **2**, 1037.

Sobel, J.D., Haim, S., Obedeanu, N., Meshulam, T. & Merzbach, D. (1977) Polymorphonuclear leukocyte function in Behçet's disease. *Journal of Clinical Pathology*, **30**, 250.

Urayama, A., Sakuragi, S., Takahashi, N., Sakai, F. & Tanaka, Y. (1974) Etiology and treatment of Behcet's disease. *Acta Societatis Ophthalmologicae Japonicae*, **78**, 1304.

Wallace, S.L., Omokoku, B. & Ertel, N.H. (1970) Colchicine plasma levels. *American Journal of Medicine*, **48**, 443.

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Case Reports

Coexistent atypical bullous pemphigoid and systemic lupus erythematosus

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SUMMARY

A vesiculobullous eruption with clinical and histological features of bullous pemphigoid developed in a 38-year-old woman with proved systemic lupus erythematosus. The patient had a sulphone responsive blistering disease that was characterized by pruritic subepidermal bullae and linear, predominantly IgA basement membrane zone deposition and IgA pemphigoid antibodies in her sera. Because both diseases are associated with immune complexes of special immunoglobulin classes, this association may not be entirely fortuitous.

During the last 10 years we have had the opportunity to evaluate various bullous diseases using immunofluorescence (IF) and other clinical procedures.

We describe here an unusual case of coexistent bullous pemphigoid (BP) and systemic lupus erythematosus (SLE). Relatively few cases of mixed immunological diseases have been reported (Jordan *et al.*, 1969; Kumar *et al.*, 1978; Miller *et al.*, 1978) and according to some authors this coexistence could be explained by common immune mechanisms (Miller *et al.*, 1978; Sams, 1970). Our patient had a sulphone responsive blistering disease that was characterized by subepidermal bullae and linear predominantly IgA basement membrane deposition.

CASE REPORT

A 38-year-old woman had a 7 year history of SLE. She was hospitalized in 1977 for diagnosis and therapy. The diagnosis of SLE fulfilled the American Rheumatology Association criteria: fever, loss of weight, arthralgia, myopathy, leukopenia and anaemia, mild proteinuria, exudative peritonitis, Raynaud's phenomenon, increased erythrocyte sedimentation rate (ESR), hypergammaglobulinaemia, positive LE-cell phenomenon. The clinical diagnosis at that time was further established by the finding of an elevated antinuclear antibody (ANF) titre and a depressed serum complement level. She responded well to a daily maintenance dose of 15–30 mg prednisone for 2 years.

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In the last 6 months of the prednisone treatment she developed a blistering eruption consisting of tense vesicles, on both erythematous and non-erythematous base, on the chest, forearms and legs The skin lesions were clinically typical of bullous pemphigoid and she was admitted to our Department in January 1979. Physical examination showed a chronically ill woman with a generalized bullous eruption primarily involving the skin of the trunk and extremities. The Nikolsky sign was negative and oral lesions were not present. She was found to have low-grade fever, fatigue, proximal muscle weakness and a widespread lymphadenopathy. The rest of the physical examination was normal.

Laboratory studies revealed leukopenia, lymphopenia and severe anaemia with a white blood cell count of $4200/\text{mm}^3$, with 76% polymorphonuclear leukocytes, 16% lymphocytes, 5% monocytes and 3% immature elements. Haemoglobin was 8·0 g/100 ml, haematocrit 26%. Protein electrophoresis revealed: albumin 3·90 g/100 ml, globulin 3·40 g% (α_1 0·25, α_2 0·75, β_1 0·66, β_2 0·23, γ 1·51). The serum immunoglobulin and complement values were: IgG 1862 mg/100 ml, IgA 189 mg/100 ml, IgM 64 mg/100 ml, C_3 49 mg/100 ml. LE-cells, rheumatoid factors and cryoproteins were found. Blood urea nitrogen, serum creatinine, creatinine clearance, chest X-ray and electrocardiogram were normal. The patient demonstrated HLA-A2, W24, B7, B12 phenotypes. Biopsy of a bulla was consistent with bullous pemphigoid, with a large subepidermal bulla and an intense perivascular inflammatory reaction mainly of round cells. Direct immunofluorescence (DIF) demonstrated a sharp linear band of IgA with less IgG and C_3 at the dermo-epidermal junction of the skin adjacent to a bulla (Fig. 1).

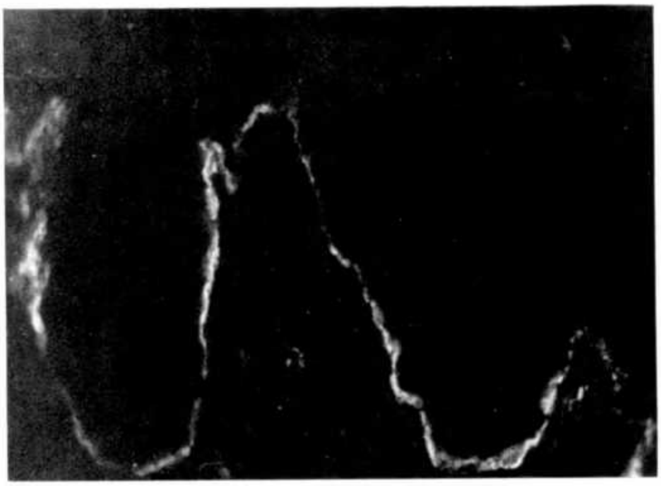

FIGURE 1. Direct immunofluorescence of the patient's skin. A brilliant, almost continuous linear fluorescence can be seen at the basement membrane region (anti-IgA, × 500).

Biopsy of a proximal muscle showed a slight focal inflammatory reaction. Granular deposition of IgA, IgG and C_3 along capillary walls was detected by direct immunofluorescence (Fig. 2).

The indirect immunofluorescence (IIF) studies of the patient's serum were positive for antinuclear antibodies with a homogeneous pattern at a titre of 1:256. At a titre of 1:32, both antinuclear and basement membrane zone antibodies (BMZ) were detected. This serum gave a positive reaction with labelled anti-IgG and anti-IgA indicating that the positive antibody classes were IgG and IgA. The preparation of skin sections, microscopy, DIF and IIF studies were as in previous papers (Cormane, Szabó & Hauge, 1970; Cormane & Szabó, 1970; Szabó, Husz & Cormane, 1977).

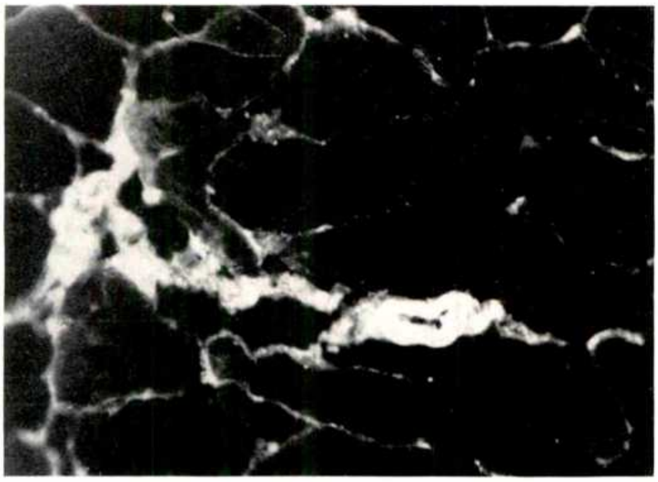

FIGURE 2. Direct immunofluorescence of patient's skeletal muscle. Note granular deposits in the vessel-wall and in the perivascular spaces (anti-IgA, × 500).

Complement-fixing antibodies were determined by *in vitro* complement IF staining (Jordon *et al.*, 1969; Klein and Burkholder, 1959), with fresh normal guinea-pig serum serving as a source of complement and FITC-labelled anti-guinea-pig C_3 (Cappel Laboratories, Inc. lot. 10184). The characteristics of the fluorescein conjugated antibody preparations were: antibody protein 1·0 mg/ml, total protein 26·4 mg/ml, F/P ratio 4:4. Controls were run with every experiment. We found that basement membrane antibodies of IgG type at lesional skin failed *in vitro* to bind C_3 from normal guinea-pig serum.

Unlike the *in vivo* fixed C_3 at the basement membrane which is common in BP (Chorzelsky & Cormane, 1968; Jordon *et al.*, 1969) BMZ antibodies in this case did not have the ability to fix complement as determined by the staining of lesional skin as well as reacting the serum with a human cryostat section (Jordon, 1976; Jordon *et al.*, 1979). It is significant that the ANA showed complement fixing ability in a higher titre and showed a mixed peripheral and speckled pattern.

Following our earlier observations on the therapeutic response to sulphone in a case with subepidermal blistering disease having IgA linear BMZ deposition (Szabó *et al.*, 1977), we added this drug to the prednisone, 100 mg/day. The therapeutic response to sulphone was excellent. Response of BP to sulpha drugs has been reported and it has been suggested that the more pyodermatous forms respond better (Jordon *et al.*, 1979).

DISCUSSION

This patient is unique clinically for several reasons. She had several humoral and cellular antibodies including lupus erythematosus factor, rheumatoid factors, antinuclear antibodies and antibodies to the basement membrane of IgA and IgG type.

Skin biopsy showed subepidermal oedema, lymphocytic infiltration and a subepidermal bulla with an absence of microabscesses in the dermal papillae. Immunofluorescence tests showed linear IgA, IgG and C_3 deposits at the BMZ. In contrast to antinuclear factors the circulating pemphigoid antibodies localized to the basement zone failed to bind guinea-pig complement *in vitro*.

This patient in addition to having well-controlled SLE had a persistent bullous disease for more than 6 months despite continuous prednisone therapy.

Patients with bullous lesions similar to our case have previously been reported under different terms, such as mixed bullous disease, linear dermatitis herpetiformis (DH), vesicular pemphigoid and atypical or polymorphic pemphigoid (Barranco, 1974; Bean *et al.*, 1976; Honeyman *et al.*, 1979; Jablonska *et al.*, 1976; Provost *et al.*, 1979).

Our analysis of the clinical features of bullous lesions is based on the major criteria (Diabowski *et al.*, 1978; Fitzpatrick *et al.*, 1971; Marks, Shuster & Watson, 1966) cited in our earlier observations (Cormane *et al.*, 1970; Cormane & Szabó, 1970). We believe that the localization of IgA, IgG and C_3, the presence of IgA and IgG anti-BMZ antibodies and the lack of an increased incidence of HLA-B8 which is found in 80–90% of patients with DH suggests that this entity is more closely related to BP than to dermatitis herpetiformis (Chorzelsky, Maciejowski & Jablonska, 1974; Diabowski *et al.*, 1978; Honeyman, 1972; Meyer & Krebs, 1976; Moncada, 1974; Peck & Lefkovits, 1066).

The fact that the SLE and BP appeared with a predominant IgA in the same patient suggests that this is the consequence of some genetic factors that can influence the code system of the humoral immune response. On this basis it can be suggested that the pathological changes of these two coexistent diseases are based on the same immune mechanism.

REFERENCES

Barranco, V.P. (1974) Mixed bullous disease. *Archives of Dermatology*, **110**, 221.

Bean, S.F., Michel, B., Furey, N. *et al.* (1976) Vesicular pemphigoid. *Archives of Dermatology*, **112**, 1402–1404.

Chorzelski, T.P. & Cormane, R.H. (1968) The presence of complement bound *in vivo* in the skin of patients with pemphigoid. *Dermatologica*, **137**, 134.

Chorzelski, T.P., Maciejowski, E. & Jablonska, S. (1974) Coexistence of pemphigus and bullous pemphigoid. *Archives of Dermatology*, **109**, 849.

Cormane, R.H., Szabó, E. & Nauge, L.S. (1970) Immunofluorescence of the skin: The interpretation of the staining of blood vessels and connective tissue aided by new techniques. *British Journal of Dermatology*, **82**, 26–43.

Cormane, R. & Szabó, E. (1970) Immuno-histopathology of the skin in pemphigoid. *British Journal of Dermatology*, **83**, 435–445.

Diabowski, J., Chorzelski, T.P., Jablonska, S. *et al.* (1978) The ultrastructural localization of IgA in skin of a patient with mixed form of dermatitis herpetiformis and bullous pemphigoid. *Journal of Investigative Dermatology*, **70**, 76–79.

Fitzpatrick, T.B., Arndt, K.A., Clark, W.H. *et al.* (1971) *Dermatology in General Medicine*, McGraw-Hill Book Co. Inc., New York.

Honeyman, J.F. (1972) The enigma of bullous pemphigoid and dermatitis herpatiformis. *Archives of Dermatology*, **106**, 21.

Honeyman, J.F., Honeyman, A.R., De La Parra, M.A., Pinto, A. & Eguiguren, G. J. (1979) Polymorphic pemphigoid. *Archives of Dermatology*, **115**, 423-427.

Jablonska, S., Chorzelski, T.P., Beutner, E.H. *et al.* (1976) Dermatitis herpetiformis and bullous pemphigoid: intermediate and mixed forms. *Archives of Dermatology*, **112**, 45–48.

Jordon, R.E., Muller, S.A., Hale, W.L. *et al.* (1969) Bullous pemphigoid associated with systemic lupus erythematosus. *Archives of Dermatology*, **99**, 17–25.

Jordon, R.E. (1976) Complement activation in pemphigus and bullous pemphigoid. *Journal of Investigative Dermatology*, **67**, 366–371.

Jordon, R.E., Lawrence, L. & Bushkell, M.D. (1979) The complement system in pemphigus, bullous pemphigoid and herpes gestationis. *International Journal of Dermatology*, **18**, 271–281.

Jordon, R.E., Mitchell Sams, J.R. & Beutner, E.H. (1969) Complement immunofluorescent staining in bullous pemphigoid. *Journal of Laboratory and Clinical Medicine*, **74**, 458–555.

Klein, P. & Burkholder, P. (1959) Die Darstellung von fixiertem Komplement mit makiertem Antikomplement. *Zeitschrift für Pathologie und Bakteriologie Schweiz*, **22**, 729.

Kumar, V., Binder, W., Schotland, E., Ernst, H., Beutner, E.H., Tadeusz, P. & Chorzelski, T.P. (1978) Coexistence of bullous pemphigoid and systemic lupus erythematosus. *Archives of Dermatology*, **114**, 1187–1190.

Marks, J., Shuster, S. & Watson, A.J. (1966) Small-bowel changes in dermatitis herpetiformis. *Lancet*, **2**, 1280–1282.

Meyer, A. & Krebs, J. Ch. (1976) Diagnostic problems associated with the coincidence of bullous disease and systemic lupus erythematosus. *Dermatologica*, **153**, 57–64.

Miller, J.F., Thomas, F., Downham, I.I. & Chapel, T.A. (1978) Coexistent bullous pemphigoid and systemic lupus erythematosus. *Cutis*, **21**, 368–373.

Moncada, B. (1974) Dermatitis herpetiformis in association with systemic lupus erythematosus. *Archives of Dermatology*, **109**, 723–725.

Peck, S.M. & Lefkovits, A.M. (1966) Bullous pemphigoid with polymyositis and co-existing contact dermatitis. *Archives of Dermatology*, **94**, 672.

Piérard, J. & Whimster, I. (1961) The histological diagnosis of dermatitis herpetiformis, bullous pemphigoid and erythema multiforme. *British Journal of Dermatology*, **73**, 253–266.

Provost, T.T., Maize, J.C., Razzak, A.A., Strauss, J.S. & Dobson, L. (1979) Unusual subepidermal bullous diseases with immunologic features of bullous pemphigoid. *Archives of Dermatology*, **115**, 150–160.

Sams, W.M., Jr. (1970) Bullous pemphigoid: is it an immunologic disease? *Archives of Dermatology*, **102**, 485.

Szabo, E., Hysz, S. & Cormane, R.H. (1977) Immunopathological findings in pemphigoid aided by developed immunofluorescence techniques. In: *Current Problems of Immunology and Their Dermatological Aspects* (Ed. by I. Rácz). Pp. 33–42. Hungarian Dermatological Society, Budapest.

Yaoita, H. & Katz, S.I. (1977) Circulating IgA antibasement membrane zone antibodies in dermatitis herpetiformis. *Journal of Investigative Dermatology*, **69**, 558–561.

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A case of lymphomatoid papulosis and systemic lymphoma*

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SUMMARY

A young man with a 4-year history of lymphomatoid papulosis subsequently developed a systemic lymphoma. Further doubt is therefore cast on the benign nature of this disorder.

The term lymphomatoid papulosis was introduced in 1968 by Macauley (1968) when he described a patient in whom an apparently benign papular eruption of long duration was characterized histologically by appearances suggestive of a malignant lymphoma. Since then lymphomatoid papulosis has been widely recognized and the subject has been recently reviewed (Macauley, 1978). The eruption consists of recurrent crops of papular or papulo-necrotic lesions often closely resembling those of pityriasis lichenoides et varolioformis acuta (Mucha-Habermann disease). Occasionally ulcerating lesions are seen which involute spontaneously to leave scars. The histological findings are of an upper and/or mid-dermal inflammatory infiltrate, amongst which are atypical lymphoid cells in sufficient numbers to suggest a malignant lymphoma; invasion of the epidermis is frequently seen. In spite of this alarming pathological appearance the disease generally pursues a benign course, often of many years duration. However, there have been three reports of the subsequent emergence of systemic lymphoma in patients with eruptions designated as lymphomatoid papulosis (Kawada *et al.*, 1969; Black & Wilson Jones, 1972; Dupont, 1973) and a further probable case was presented to the Section of Dermatology of the Royal Society of Medicine by Dr P.M.Dowd and Dr D.Dowling Munro in November 1979. We wish to report a further example of systemic lymphoma in association with this spectrum of skin disorders.

CASE REPORT

A man, then aged 21, first presented to the Skin Department, North Staffordshire Hospital Centre, Stoke-on-Trent, in August 1977. On examination, there was a papular eruption on the arms and legs, many of the lesions being varioliform. General examination was normal. A diagnosis of pityriasis lichenoides et varioliformis acuta was made.

* This case was presented to the British Society for Dermatopathology, Glasgow, January 1980.

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Investigations

The following were either normal or negative: full blood count (FBC), ESR, 12-function biochemical screen, anti-nuclear antibody, latex test for R.A. factor, VDRL and chest X-ray. IgG antibody titre to Epstein-Barr Virus (EBV) capsid antigen was 1 in 10.

The patient was treated unsuccessfully with once weekly exposure to UVB for 3 months and then failed to attend for further follow-up.

He re-attended in June 1979. He had continued to suffer from recurrent crops of papules, chiefly on the limbs, some of which had ulcerated and resolved leaving scars. In September 1978 he had noticed a painless swelling in the left groin which gradually enlarged with ulceration of the overlying skin.

On examination there were papular lesions on the limbs similar to those seen in 1977 although the proportion of lesions being varioliform was much diminished. On the buttocks and thighs there were several small disc-shaped scars of previous ulcerations. In the left inguinal region there was a diffuse firm mass and three necrotic ulcers of the overlying skin (Fig. 1). The right inguinal lymph nodes were enlarged. General examination was otherwise normal.

Biopsies were taken from three papules on the limbs and from the edge of an ulcer in the groin. The right inguinal nodes were biopsied under general anaesthetic.

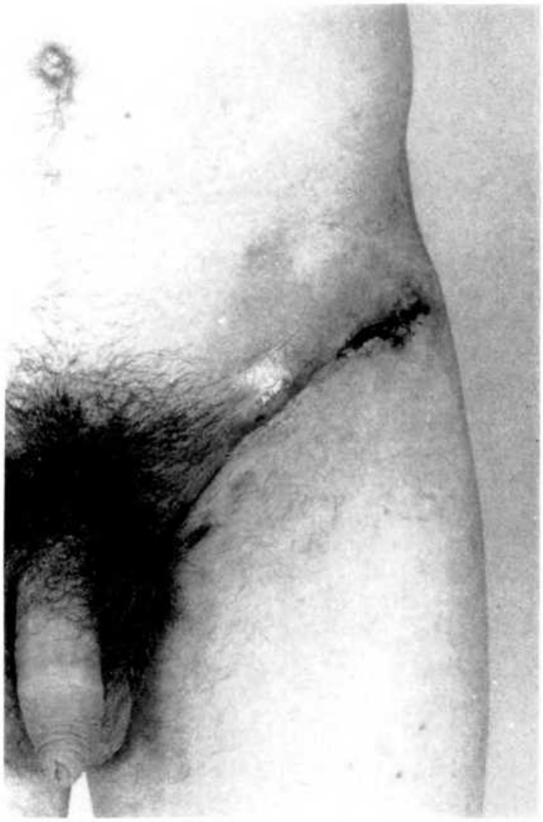

FIGURE 1. Ulcerating lesions in the left groin. Papular lesions can also be seen on the thigh and abdomen.

The following investigations were either normal or negative: FBC, ESR, biochemical screen, VDRL, sternal marrow biopsy and chest X-ray. Antibody titre to EBV was now 1 in 160. A bipedal lymphogram showed bilateral enlargement of inguinal, iliac and para-aortic nodes with a fine reticular foamy appearance suggestive of reticulosis.

Histopathology

The biopsies were embedded in paraffin wax and in plastic and stained with haematoxylin and eosin.

All three papules were similar but particular features required step sections to be shown. There was mild acanthosis and patchy parakeratosis, although in some levels this formed a complete parakeratotic scale. There was a dense nodular infiltrate predominantly in the mid-dermis but also extending, mainly in a perivascular distribution, into the upper dermis (Fig. 2). The infiltrate consisted of small hyperchromatic, frequently angular, lymphocytes and scattered amongst these, sometimes in clusters, large mononuclear cells with irregular chromatin, one or two nucleoli and indistinct cytoplasm (Fig. 3). Mitotic figures amongst this latter population of cells were frequent. In some levels, small numbers of hyperchromatic mononuclear cells were seen invading the epidermis. The papules differed in their histological appearances from those of pityriasis lichenoides et varioliformis acuta and from the appearances more often associated with lymphomatoid papulosis by the absence of gross interface dermatitis.

The biopsy specimen from the edge of the ulcer showed acanthosis, in places of psoriasiform configuration, with a dense upper and mid-dermal infiltrate composed mainly of large mononuclear cells (Fig. 4). The nuclei tended to be bean-shaped, sometimes to a marked degree, and ring forms were occasionally seen. The nucleoli were large and often multiple and the chromatin pattern tended to be vesicular. Mitotic figures were once again frequent. At the edge of the area of ulceration these cells exhibited marked epidermotropism. Deeper down in the lesion there were patchy foci of non-specific inflammatory cells.

Four lymph nodes from the right groin were examined. All, to some extent, showed the typical reactive changes seen after lymphangiography but the largest node, in addition, contained a nodule

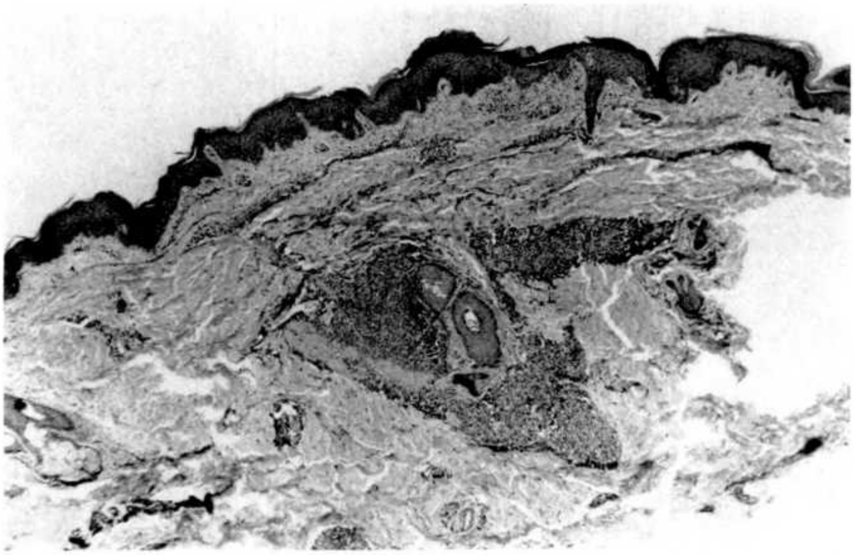

FIGURE 2. A papule showing intense mid-dermal nodular infiltrate and parakeratosis (× 24).

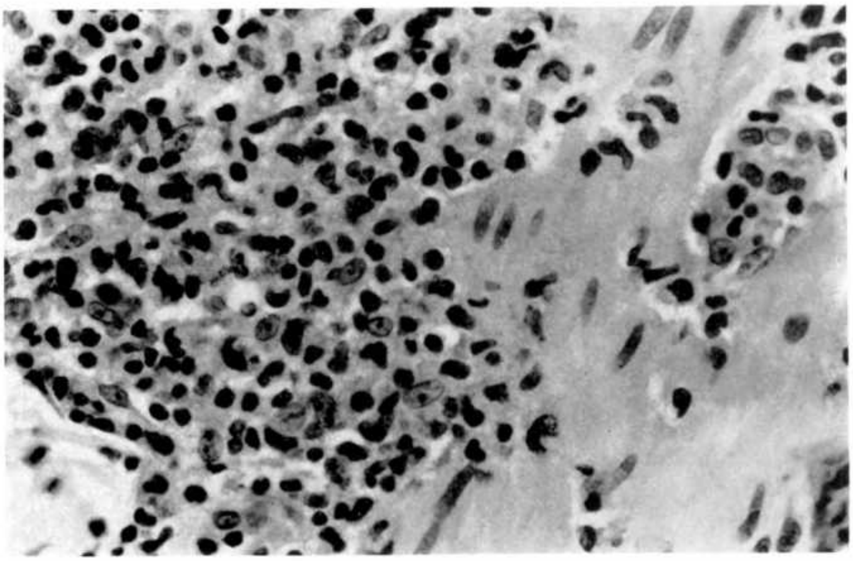

FIGURE 3. Papule showing small hyperchromatic cells with scattered large mononuclears. An arrector pilorum muscle is infiltrated (× 140).

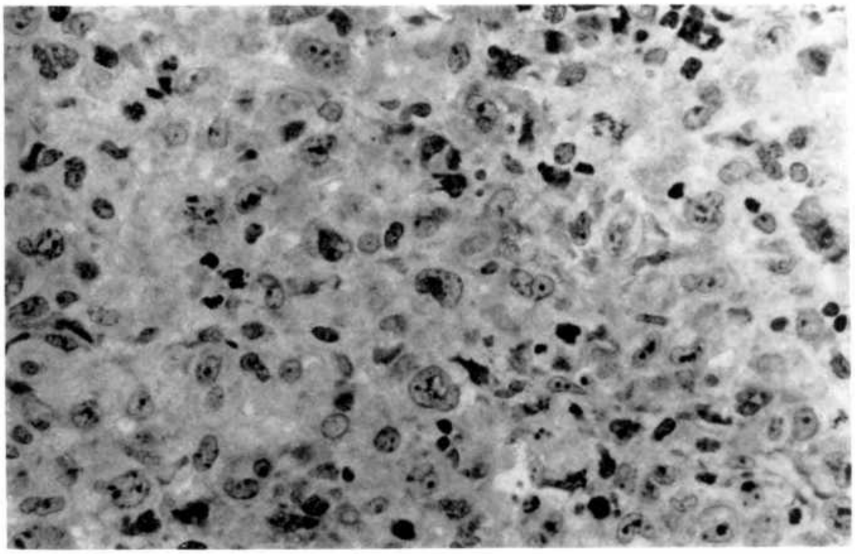

FIGURE 4. From edge of groin ulcer. High power view of infiltrate showing large atypical mononuclear cells (plastic section, × 350).

composed of cells which were virtually identical to those seen in the groin ulcer and to the large mononuclear cell population in the skin lesions (Fig. 5). The only deviation from their appearance in the skin was that in the lymph node the cells were, in places, tending to be spindle-shaped and occasional bi-nucleate forms were observed. The overall appearance, however, was of a non-Hodgkin's large-cell lymphoma.

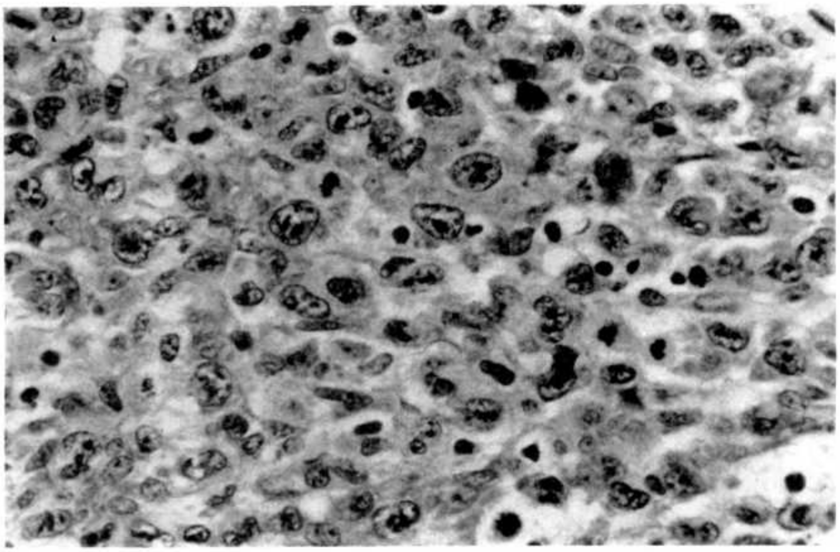

FIGURE 5. Lymphomatous nodule in lymph node. Note similarity of cells to large mononuclear cells in Figs 3 and 4 (× 350).

The patient has been treated with standard courses of quadruple therapy (prednisolone, procarbazine, mustine, vincristine) with rapid resolution of the ulcerating lesions and of the mass in the left groin. For some time he continued to develop crops of papules but at the time of writing he has been clear of all lesions for eight months.

DISCUSSION

The term lymphomatoid papulosis has been adopted since Macauley's original report to describe a recurrent self healing papular eruption, sometimes resembling pityriasis lichenoides et varioliformis acuta, which has histological features suggesting a lymphoma. We feel our case fulfils these criteria.

Black & Wilson Jones (1972) studied the clinical and histological features of fifteen cases of 'lymphomatoid' pityriasis lichenoides. In most of their cases the abnormal lympho-mononuclear infiltrate was concentrated in the upper dermis although in some larger lesions it extended to the lower dermis and even to the hypodermis. Epidermal invasion by the atypical and other mononuclear cells was a prominent feature in most biopsies although this varied considerably in degree depending on the stage, size and type of the lesion. The number of atypical cells present also varied and in smaller papules having an adherent scale were sometimes absent. These authors proposed that lymphomatoid papulosis is essentially a variant of pityriasis lichenoides in which the proportion of atypical cells in the infiltrate suggests a malignant lymphoma.

In our case the infiltrate in all the papular lesions biopsied is predominantly mid-dermal and epidermotropism, although present, is not a prominent feature. The ulcer in the groin does indeed show the more typical histology of lymphomatoid papulosis but direct extension from underlying lymph node disease could not be excluded. In his recent review Macauley (1978) cites a number of cases, which although behaving clinically like lymphomatoid papulosis and having the histology of a malignant lymphoma, exhibit considerable variation in cellular morphology, cell ratios and distribution of cells. He proposes the term 'rhythmic paradoxical eruptions' to encompass this broader view of lymphomatoid papulosis and our case could be regarded as falling within this spectrum.

Although lymphomatoid papulosis generally runs a benign course our patient is now the fourth reported case of the subsequent development of systemic lymphoma. The cellular morphology strongly suggests that the lymph node disease is the same as that in the skin. Whether the disease process originates in the skin or the cutaneous lesions are merely a manifestation of pre-existing systemic disease is not yet clear. The long interval between onset of skin lesions and evidence of systemic involvement—four years in our case and even longer in previous reports—would seem to support the former hypothesis.

What is the explanation for the rise in antibody titre to EBV from 1:10 in 1977 to 1:160 in 1979? EBV infection is common, especially in this age group, and the titre change may of course reflect such a recent incidental infection. Elevated EBV antibody titres have been reported in Hodgkin's disease, non-Hodgkin's lymphoma and chronic lymphatic leukaemia. Ten Napel *et al.*, (1978) showed that in Hodgkin's disease elevated antibody titres to EBV were associated with low or negative specific lymphocyte reactivity to EBV. Impairment of cellular immunity, such as occurs in Hodgkin's disease, may allow enhanced EBV antigen proliferation in previously infected B-lymphocytes resulting in increased antibody production. In our patient we may be seeing indirect evidence of impaired T-lymphocyte function which has allowed dissemination of a lymphoma previously confined to the skin. The third possibility is that EBV is more directly involved in pathogenesis. Boss *et al.*, (1978) produced preliminary evidence to suggest that pityriasis lichenoides may result from EBV infection but Macauley (1978) states that in co-operation with Henle he examined the sera of twenty one patients with pityriasis lichenoides and lymphomatoid papulosis and was unable to detect significant differences in EBV antibody titres from what might be expected in a normal population. EBV is known to have the capacity to transform human B-lymphocytes and to be oncogenic for certain non-human primate lymphoid cells and is strongly implicated in the pathogenesis of both Burkitt's lymphoma and nasopharyngeal carcinoma. It is tempting to speculate that EBV has played a similar oncogenic role in this case. Should further tissue become available the demonstration of the viral genome within the lymphoma cells would provide supportive evidence for this hypothesis.

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REFERENCES

BLACK, M.M. & WILSON JONES, E. (1972) 'Lymphomatoid' pityriasis lichenoides; a variant with histological features simulating a lymphoma. *British Journal of Dermatology*, **86**, 329.

BOSS, J.M., BOXLEY, J.D., SUMMERLY, R. & SUTTON, R.N.P. (1978) The detection of Epstein-Barr virus antibody in 'exanthematic' dermatoses with special reference to pityriasis lichenoides: a preliminary survey. *Clinical and Experimental Dermatology*, **3**, 51.

DUPONT, A. (1973) Transformation maligne très tardive d'une réticulose papuleuse à évolution prolongée. *Annales de Dermatologie et de Syphiligraphie*, **100**, 141.

KAWADA, A., ANEKOJI, K., MIYAMATO, M., NAKAI, T. & MORI, S. (1969) Unusual manifestation of malignant reticulosis of the skin: cutaneous lesion simulating parapsoriasis guttata. *Dermatologica*, **138**, 369.

MACAULEY, W.L. (1969) Lymphomatoid papulosis: a continuing self healing eruption, clinically benign—histologically malignant. *Archives of Dermatology*, **97**, 23.

MACAULEY, W.L. (1978) Lymphomatoid papulosis. *International Journal of Dermatology*, **17**, 204.

TEN NAPEL, CHR., H.H., THE, T.H., VAN EGTEN-BIJKER, J., DE GAST., G.C., HALIE, M.R. & LANGENHUYSEN, M.M.A.C. (1978) Discordance of Epstein-Barr virus specific humoral and cellular immunity in patients with malignant lymphomas: elevated antibody titres and lowered *in vitro* lymphocyte reactivity. *Clinical and Experimental Immunology*, **34**, 338.

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Localized cold reflex urticaria

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SUMMARY

A patient is presented who developed small weals in the vicinity but not at the place of contact with cold. The lesions occurred only on the extremities and during the cold season. There were no abnormal laboratory findings and no measurable histamine or ECF levels in the venous effluent of the affected region. Skin reactivity to acetylcholine was normal and was decreased in response to histamine and compound 48/80. This type of physical urticaria may represent a localized variety of the extremely rare cold reflex urticaria.

Among the physical urticarias, the so-called cold urticarias are the second most frequent type (Illig & Kunick, 1969). Typically, patients with this disease develop redness and wealing at the site of contact with cold objects or with cold winds. A number of types of cold urticaria have been described, including some that are secondary to specific systemic diseases and some that are familial (Warin & Champion, 1974). In this case report we describe a patient with a type of cold urticaria that does not seem to fit into any of the categories of that disease known so far.

HISTORY

A 23-year-old male medical student presented with a one year history of itching and urtication on exposure to the cold. After riding a bicycle or after exercising in the cold, itching and redness developed on the exposed parts of the extremities and spread to the upper thighs and arms within the next few minutes, without ever involving trunk or head. On close examination, the student had noted pinhead-size weals in the mildly itching, red areas. There was no associated systemic discomfort, and the wealing and itching had generally ceased within 2–3 h after exposure. During the cold season the symptoms recurred almost daily but disappeared when the weather became warmer.

There was no history of prior illness, of taking medications, or of allergies. The family history was positive for hay fever on the father's side.

PHYSICAL FINDINGS

On physical examination, the patient presented no gross pathological findings of the skin or the

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internal organs. The following laboratory values were obtained at the time he had his symptoms: haemoglobin 15·5%; WBC 3000/mm^2, with 2% eosinophils, 4% monocytes, 57% neutrophils and 37% lymphocytes. The total serum protein was 76·0 g/l, with a normal electrophoresis. Cryoglobulins, ANF and syphilis serology were negative. The total serum IgE was 101 u/ml. Serum liver enzyme levels and ESR were not raised.

A plastic bag filled with ice was placed on the forearm covering an area of about 5 × 5 cm, and this site and the surrounding skin were observed for the development of redness or urtication. Patches of redness developed in the vicinity, but not at the site of contact, within 2–5 min and small weals, 1–5 mm in diameter, were noted in the same region within seconds thereafter (Fig. 1). A confluent weal at the site of contact, as is typical for cold urticaria, was never observed. The reaction waned 20–40 min after it had begun.

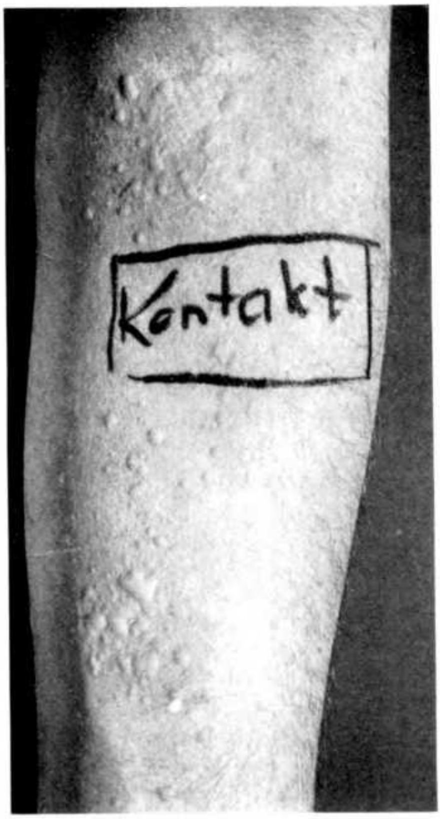

FIGURE 1. 1–2 mm size weals, at some places confluent, on localized patches of erythema on the left forearm of the patient. A bag with ice had been applied in the area marked 'kontakt'.

Stroking of the skin of the back with mild pressure produced an almost immediate wealing with a red flare. Application of a small electric current, as is used for epilation, also produced punctate localized wealing. Rigorous exercise on a bicycle, which is used in our clinic routinely for the diagnosis of cholinergic urticaria, did not produce the typical generalized small weals.

Histamine and eosinophil chemotactic factor (ECF) were measured for up to 30 min in the venous effluent of both forearms. A large ice bag was applied to one of the forearms to induce urtication.

Neither ECF, assayed in Boyden chambers as previously described (Czarnetzki, Konig & Lichtenstein, 1976), nor histamine, measured in acid precipitated serum by a spectrofluorometric method (Shore, Burkhalter & Cohn, 1959), increased in the blood during the test period.

SKIN TESTING

The following substances were injected (0·05 ml) intracutaneously on the skin of the back: pyrogen-free isotonic saline, 0·01% histamine, 1% acetylcholine, and 0·1% compound 48/80. The diameter (in mm) of weal and erythema were measured 10 min later in the largest and smallest dimensions. Table 1 shows the measurements for the patient, and the mean and standard deviations of the skin reactions obtained by the same method with ten normal volunteers. The response to histamine and to a histamine liberator, compound 48/80, was reduced in the patient, but the reaction to acetylcholine did not differ from that of controls.

TABLE 1. Size of weals (W) and erythema (E) after intradermal injection of 0·05 ml of isotonic pyrogen-free saline, 0·01% histamine, 1% acetylcholine and 0 01% compound 48/80. The numbers are the products of the largest and smallest diameter, measured in mm. The patient's values are compared with the mean values and standard deviations, obtained by the same method with ten normal volunteers.

Stimulus	Patient	Controls
NaCl	(W) 36·0	(W) 50·2 ± 20·6
	(E) 0	(E) 0 ± 0
Histamine	(W) 48·0	(W) 190·5 ± 123·4
	(E) 169 0	(E) 3207·6 ± 1890·1
Acetycholine	(W) 121·0	(W) 73·0 ± 28 0
	(E) 1024 0	(E) 982·0 ± 328 0
Compound 48/80	(W) 100·0	(W) 152·8 ± 87·9
	(E) 225·0	(E) 1563·3 ± 782 4

COURSE OF THE ILLNESS

One week after these tests had been conducted the weather had become slightly warmer and there was a delay in the production of the reflex urticaria after exposure to cold. After one further week of continuing warm weather, the patient had not noticed any recurrence of his symptoms, and the reflex urticaria could not be reproduced even after application of cold for more than half an hour. The dermographism (urticaria factitia) after application of a scratch with light pressure had disappeared as well.

DISCUSSION

The clinical picture presented here must be grouped with the so-called physical urticarias because of the nature of the eliciting stimulus, the small size of the lesions and the brief latency period prior to development of the weals. We have chosen the name localized cold reflex urticaria because, during provocation in the hospital and on natural exposure in normal life, there was never a generalization of the rash to the trunk or the head. Instead, the lesions developed in the vicinity of the contact area and in a pattern suggestive of involvement of the nervous reflex arches. Direct involvement of nerves in the production of the lesion could not be proven, however, and the lesion might also be explained by radiation of cold from the ice bag. Such a mechanism would not explain the lack of involvement of the area of testing, even after rewarming. By its physical appearance, the distribution of the lesions and the negative laboratory findings, the clinical picture does not fit with the essential or the secondary types of cold contact urticaria (Warin & Champion, 1974). The diagnosis of a typical cholinergic urticaria was ruled out by the negative exercise test.

Illig (1973) has described a patient who developed small, papular weals on exposure to cold air or cold water. In contrast to our patient, this urtication was generalized, as is observed with cholinergic urticaria. Another patient, described by Petres & Kunick (1978), developed a generalized erythema after local exposure to cold. Weals were found only at the site of contact. An intriguing observation, because of the possible clue to the pathogenesis of the disease, was also made by Gerhard & Schopf (1974) who reported localized cold contact urticaria at sites of previous intracutaneous testing. Nothing happened at places distant from the test area. Our patient thus does not fit with any of these reported cases.

There are a few other observations of interest with our patient, such as the occurrence of marked dermographism only during the time period when he also had his cold urticaria. The decreased responsiveness to skin testing with histamine and compound 48/80 which we have not noted on testing ten normals or patients with cholinergic urticaria is another striking feature. The failure to demonstrate a transient rise in serum histamine and ECF on exposure to cold, as previously reported with cold and cholinergic urticaria patients (Kaplan *et al.*, 1976; Soter, Wasserman & Austen, 1976), may be due to the localized and mild nature of the reaction in our patient.

In a large percentage of patients with physical urticaria, a factor, most likely IgE, has been found in the serum which can passively transfer their disease to healthy volunteers (Warin & Champion, 1974). We have not done this test for obvious ethical reasons. One might speculate, though, that the close association of his symptoms with the cold season could be due to a special factor that is formed on exposure to cold. This substance could in turn directly induce mast cell release or act via IgE antibody on specifically sensitized mast cells. The restriction to the extremities with sparing of the equally exposed face suggests that localized rather than circulating factors are responsible for the development of the lesions.

Physical urticarias frequently appear for no obvious reason and, after a varying time period, stop vexing the patient, again without any apparent reason. We have no clue what factors might have induced the appearance of the symptoms with our patient. Fortunately, discomfort due to the lesions was localized, minor and confined to one season of the year. This may also be one reason why this type of urticaria has not come to the attention of physicians before.

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REFERENCES

CZARNETZKI, B.M., KÖNIG, W. & LICHTENSTEIN, L.M. (1976) Antigen-induced eosinophil chemotactic factor (ECF) release by human leukocytes. *Inflammation*, 1, 201.

GERHARD, H.J. & SCHÖPF, E. (1974) Uber ein Kobnerphänomen bei sog. Kaltwasser- bzw. Kaltluft-Urtikaria. *Hautarzt*, 25, 197.

ILLIG, L. (1973) Pnysical urticaria, its diagnosis and treatment. In: *Current Problems in Dermatology* (Ed. by J.W.H. Mali), Vol. 5, p. 79. Karger, Basel.

ILLIG, L. & KUNICK, I. (1969) Klinik und Diagnostik der physikalischen Urticaria. *Hautarzt*, **20**, 167.

KAPLAN, A.P., GRAY, L., SHAFT, R.E., NORAKOVA, Z. & BEAVEN, M.A. (1975) *In vivo* studies of mediator release in cold urticaria and cholinergic urticaria. *Journal of Allergy and Clinical Immunology*, **55**, 394.

PETRES, J. & KUNICK, I. (1978) Ungewöhnliche Kaltwasser- und Kaltluftkontaktuberempfindlichkeit mit fortgeleiteter reflexähnlicher Erythembildung. *Hautarzt*, **29**, 420.

SHORE, P.A., BURKHALTER, A. & COHN, V.H., Jr. (1959) A method for the fluorometric assay of histamine in tissues. *Journal of Pharmacology & Experimental Therapeutics*, **127**, 182.

SOTER, N.A., WASSERMAN, S.I. & AUSTEN, K.F. (1976) Cold urticaria: release into the circulation of histamine and eosinophil chemotactic factor of anaphylaxis (ECF-A) during cold challenge. *New England Journal of Medicine*, **294**, 687.

WARIN, R.P. & CHAMPION, R.H. (1974) *Urticaria.* W.B. Saunders Co., London.

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Encephalocraniocutaneous lipomatosis: a new neurocutaneous syndrome

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SUMMARY

Encephalocraniocutaneous lipomatosis is a congenital neurocutaneous disorder with the distinguishing histopathological features of dysgenesis and neoplasia of the adipose tissue. The dominant clinical features of the syndrome include convulsions beginning in infancy, mental retardation, and unilateral cutaneous and ophthalmological lesions with ipsilateral cerebral malformations. A patient with this rare disorder of ectomesodermal dysgenesis has been studied in order to classify clinically and histologically the associated skin lesions. To our knowledge, this is the fourth case of encephalocraniocutaneous lipomatosis reported in the English literature.

Encephalocraniocutaneous lipomatosis (ECCL) was first described in 1970 by Haberland & Perou as a new example of ectomesodermal dysgenesis (Haberland & Perou, 1970). They reported a patient who exhibited severe mental retardation, convulsions beginning in infancy, and unilateral eye (choristomas) and skin lesions (lipomas and connective tissue naevi) associated with ipsilateral cerebral malformations. Post-mortem histological examination revealed multiple lipomatous tumours in tissues derived from the embryonic ectodermal and mesodermal layers. Although some of the features found in the patient were common to other neurocutaneous disorders, the total constellation of findings suggested a new syndrome.

Two additional patients were described by Fishman and co-workers in 1978, but unlike the original patient, they had only mild mental retardation (Fishman *et al.*, 1978). All three patients described in the literature were males and there was no suggestion of familial transmission. The present report describes a female with ECCL who exhibits mild mental retardation, multiple angiofibromas and bilateral skin and eye lesions. Contrast studies and computerized axial tomography, however, reveal unilateral cerebral malformation.

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CASE HISTORY

A 3-week-old girl (born October 10, 1977) was admitted to The Children's Hospital Medical Center, Boston, Massachusetts, on November 8, 1977, because of increased head circumference and 'growths on the eyes and face'.

At birth the infant weighed 3·35 kg and was the product of a Gravida III, Para I, 27-year-old mother. The pregnancy was full term and complicated by otitis media in the third trimester treated with ampicillin. The delivery was an uncomplicated vaginal birth using low forceps under spinal anaesthesia.

At 1 h of age, red 'growths' were noted on the bulbar conjunctivae. Similar masses became apparent on the skin around the eyes and on the scalp during the first day. The left mid-frontal scalp had a 5 cm area of alopecia. The facial lesions receded in size, and the colour of the eye lesions changed from red to flesh tones over the first several weeks. The infant was breast fed, but failed to gain weight.

When admitted to the hospital at 3 weeks of age, the infant weighed 3 kg (less than 3rd percentile), the height was 52 cm (25th percentile) and the head circumference 52 cm (9th percentile). She was pale, irritable and wasted. An 8 mm soft tumour with the colour and consistency of fatty tissue was prominent over the left zygomatic area. There were multiple 3–5 mm pink soft papules arising from the outer canthi of both eyes and multiple soft flesh-coloured nodules on the scalp. A 5 × 10 cm irregular smooth patch of alopecia was present on the left frontal area of the scalp, with telangiectases below the skin surface. Inferior to the left orbit there were several 3 mm raised papules with central erythema, each blanching with pressure and refilling from the centre. There were also numerous raised lesions approximately 1 mm in diameter on the bulbar conjunctiva. The remainder of the physical examination was remarkable for hydrocephalus and generalized wasting. The liver, spleen and lymph nodes were not significantly enlarged, and the cardiac and fundoscopic examinations were normal.

An EEG was abnormal due to left posterior quadrant sharp and spikey activity with a component of 0·5–1 Hz delta slowing. Computerized axial tomography revealed an obstructed, grossly enlarged left lateral ventricle. A ventriculogram revealed communication between a porencephalic cyst and the ventricle.

Family history revealed that the mother's sister died at 2 weeks of age with 'water on the brain,' and the father's sister had a stillborn infant with no known abnormalities.

At the age of 3 months, the infant had a ventriculo-peritoneal shunt placed because of hydrocephalus and a porencephalic cyst. She has had several admissions since for shunt revisions due to malfunction. After a shunt revision procedure at 3 months, the infant had the first of many convulsions, each of which was usually preceded by a loud cry and followed by brief episodes (lasting seconds) of cyanosis and a post-ictal state of 'sleeping'.

Neurological examination at the age of 6 months revealed bilateral motor impairment which was greater on the right side. Sensation, mental status, and cranial nerve function were normal.

Since the age of 6 months the child has had difficulty with convulsions. Presently at the age of 2 years, she is taking Dilantin and phenobarbitone, having approximately one convulsion per week, characterized by episodes of staring followed by sleep.

The child's physical examination at age 2 years showed the same soft tissue papules and tumours 2 mm to 3 cm in diameter about the (L) cheek, both eyelids, both bulbar conjunctivae, left forehead, and neck (Figs. 1–3). Hairless smooth plaques were present over two-thirds of the (L) parietal scalp and one-third of the (R) parietal scalp, with normal skin markings but no hair follicles seen in the areas of alopecia. The skin lesions were thought to be stable without progression. Lymph nodes, spleen and liver still showed no enlargement. Hydrocephalus and spasticity of the right arm and right

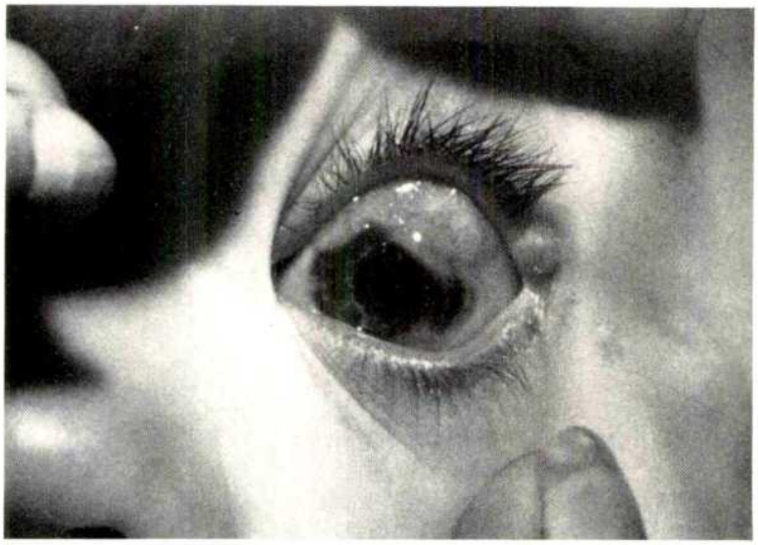

FIGURE 1. Fleshy soft plaque on left bulbar conjunctiva obscuring normal outline of iris. A soft papule on left upper lid margin, representing angiofibroma (age 2 years).

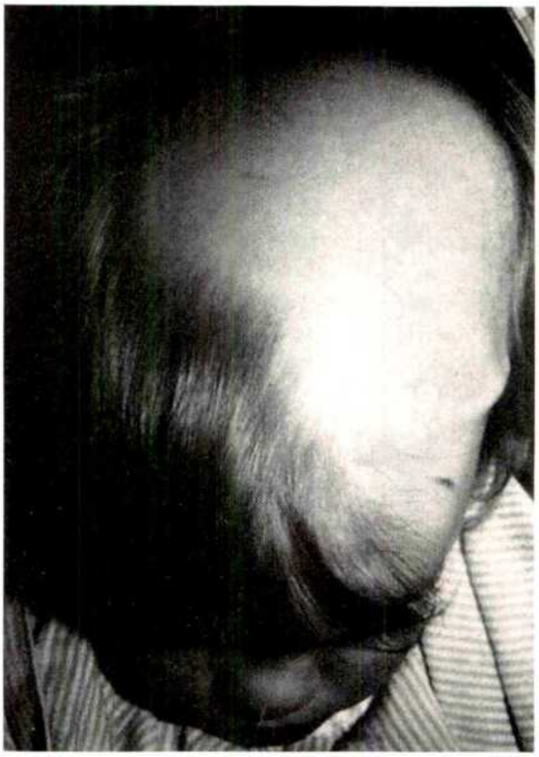

FIGURE 2. Cutaneous lipomatosis: hairless plaque involving most of left and part of right side of the scalp (age 2 years).

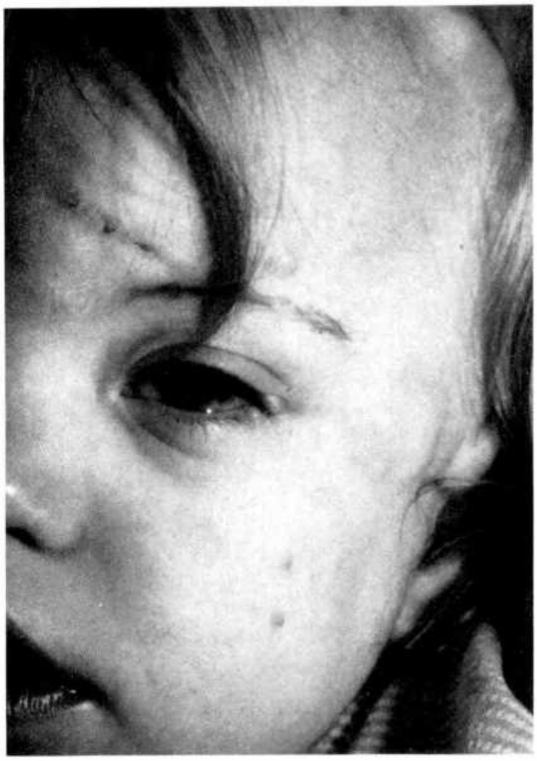

FIGURE 3. Cutaneous lipomatosis and angiofibromas: fleshy soft colourless plaque in zygomatic region and scattered papules over left forehead, cheek and upper eyelid (age 2 years).

leg without progressive neurological signs characterized her most recent neurological findings. Computerized axial tomography performed at the age of 20 months showed a large cystic lesion in the left hemisphere with hemiatrophy. Laboratory examination at 20 months revealed: haematocrit 36·1%, Hb 11·9 g/dl, WBC 8,700/mm^3 (polys 73, bands 3, lymphs 13, monos 11%), 320,000/mm^3 platelets; urinalysis sp. gr. 1003, pH 7, no protein or glucose, 2–5 WBC/hpf; blood urea nitrogen 6 mg%, glucose 80 mg%, sodium 135 mg/l, potassium 4·0 mg/l, chloride 101 mg/l, total CO_2 26 mol/l.

At the age of 2 years, the girl's development was noted to be delayed, and she was just beginning to take several steps alone. She used single words, and had difficulty with fine motor coordination and balance. Her weight at 2 years was 11·14 kg (25th percentile) and her height 87·63 cm (50th percentile). She has had multiple hospital admissions because of shunt complications, including ventriculitis in October, 1977. The convulsions have been controlled moderately well with phenobarbitone and Dilantin.

HISTOPATHOLOGY

Histopathology of papules on the face and scalp revealed a cutaneous hamartoma in which there was a dermis of irregularly shaped collagen fibres of smaller diameter than those of normal reticular dermis. These abnormal fibres extended into the subcutis and formed large fibrous septa associated with increased amounts of subcutaneous fat (Fig. 4). This fat focally extended into the upper reticular

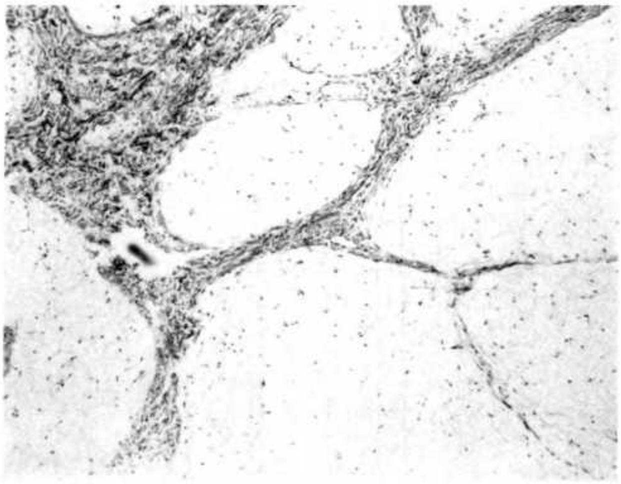

FIGURE 4. Increased amounts of fat intermixing with irregularly shaped collagen fibres of reticular dermis obscuring the normal architecture of the reticular dermis-subcutaneous junction (H & E, × 100).

dermis (Fig. 5). The hamartoma affected the lower reticular dermis and the subcutis which together formed a fibrolipoma. In the abnormal dermis there was a sparse, diffuse inflammatory cell infiltrate which contained a strikingly large number of mast cells, confirmed with Giemsa's reagent. Examination of a papule from the temple exhibited fine dermal fibrosis in a lamellar array around appendageal structures, associated with slight capillary hyperplasia (Fig. 6). These changes resembled an angiofibroma.

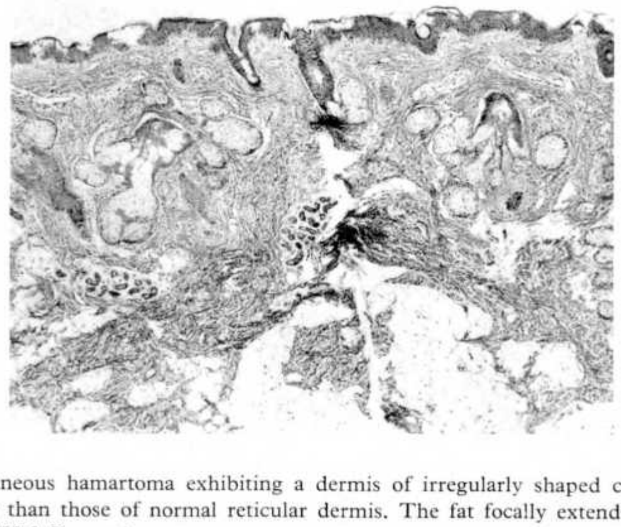

FIGURE 5. Cutaneous hamartoma exhibiting a dermis of irregularly shaped collagen fibres of smaller diameter than those of normal reticular dermis. The fat focally extends into the upper reticular dermis (H & E, × 55).

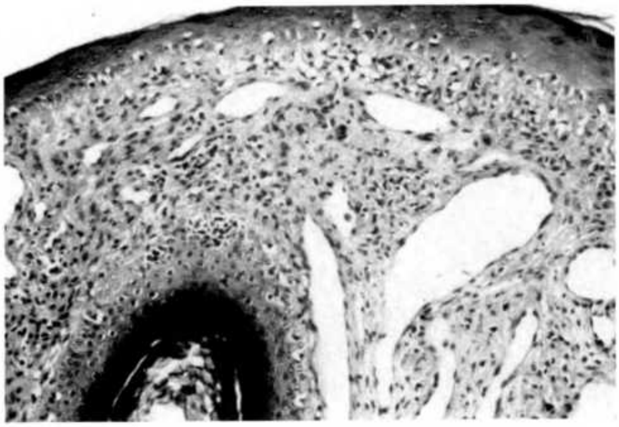

FIGURE 6. Histology of papule from the temple showing fine dermal fibrosis in lamellar array around appendageal structures, associated with capillary dilatation and hyperplasia (H &E × 250).

DISCUSSION

Previous studies of patients with ECCL have emphasized male sex, delayed or grossly abnormal fibrolipomas, connective tissue naevi, alopecia in the area of scalp lesions, pterygium-like choristomas of the eyes, and bony protuberances of the skull. The patient in this report broadens the clinical spectrum of ECCL to include the female sex and bilateral skin lesions on the face and scalp. A new finding, angiofibromas, has not been specifically noted in previous reports.

Also unique in our patient were the central nervous system findings. Although our patient had bilateral lipomatosis of the skin, cranial studies exhibited unilateral cerebral hemispheric atrophy with a dilated ventricular system and porencephaly. The extent of these malformations was not as severe as those described in previous reports, such as the patient described by Haberland in which unilateral atophy of the cerebral hemisphere associated with cerebral calcification on the side of the skin lesions was accompanied by a congenital mantle defect of the temporal lobe, partial opercularization of the insula, micropolygyria of the entire hemisphere, a dilated ventricular system, a lipoangiomatous malformation of the meninges over the involved hemisphere, and several lipomas in the middle fossa, leptomeninges and adjacent to the trigeminal nerve on the same side (Haberland & Perou, 1970).

The extension of the lipomatosis to the skull, eye, and viscera in ECCL closely parallels the angiomatous involvement of these sites in Sturge-Weber syndrome; however, in Sturge-Weber syndrome there is no blastomatous angiomatosis, and the mesodermal dysgenesis is usually limited to the malformation of blood vessels (Alexander & Norman, 1960). In ECCL the cutaneous and leptomeningeal lipomatosis occurs in conjunction with multiple lipomas of the nervous system.

Another disease that superficially resembles ECCL and is often associated with neurological abnormalities is the epidermal naevus syndrome (Solomon & Esterly, 1975), in which the skin lesions are histologically different from ECCL, and although unilateral may occasionally involve the midline of the face. Associated cerebral malformations are occasionally responsible for convulsions and mental retardation in the extensive linear naevus sebaceus syndrome (Fenstein & Mims, 1962) and they include unilateral ventricular enlargement, symmetrical hydrocephalus *ex vacuo*, unilateral ventricular dilatation, arachnoid cysts and filling porencephaly with non-functioning of the major cerebral venous sinuses (Chalub, Volpe & Gadi, 1975). Early emotional and neurological development may be normal in the linear naevus sebaceous syndrome, despite the presence of cerebral abnormalities detected by computerized tomography (Marks & Tomasovic, 1980). The hamartomatous development of epidermis in the epidermal naevus syndrome and the anomolous connective tissue proliferation in ECCL illustrate the close developmental relationship between the neuro-ectoderm and connective tissue mesoderm. The defect in ECCL appears to involve embryonic ectodermal and mesodermal elements at a critical period of development, but the pathogenesis remains unknown.

Lesions that resemble lipomatous connective tissue naevi clinically occur in the focal dermal hypoplasia syndrome (Goltz *et al.*, 1970). The striking differences include the histological findings of hypoplasia of dermis and herniation of fat into the superficial papillary dermis. Arborescent papillomas occur in periorificial sites in this syndrome, requiring surgical removal because of ulceration and bleeding. Osteopoikilosis, an asymptomatic bony abnormality characterized by multiple round or oval densities on X-ray examination of long bones, pelvis, hands and feet, has been associated with multiple symmetrical connective tissue naevi or so-called dermatofibrosis lenticularis disseminata (Schorr *et al.*, 1972; Ragne & Wood, 1970). Hypopigmented connective tissue naevi have been reported in association with macrodactyly and hemihypertrophy (Temtamy & Rogers, 1976). Superficial naevus lipomatosus (SNL) is an uncommon lesion present at birth, characterized by solitary or multiple superficial plaques without systemic abnormalities. Extensive lesions known as underlying naevus lipomatosus (UNL) have been associated with folding of the skin, abnormalities of the feet, hemiplegia, low-set ears, abnormalities detected of chromosome 11, hemihypertrophy of the extremities, mental retardation and microcephaly (Gardner, Miller & Lowney, 1979; Ross, 1969). The Shagreen patch of tuberous sclerosis represents a type of connective tissue naevus and again demonstrates the developmental relationship between mesoderm and ectoderm (Kobayasi *et al.*, 1973). The majority of solitary small naevi of epidermal, connective tissue or naevus cell origin are not associated with any underlying disease.

It has been postulated that lipomas develop from the connective tissue apparatus of the newly formed blood vessels, fat cells first appearing during the 3rd fetal month (Scherer, 1935). It has been further reasoned that inborn or environmentally induced enzymatic or metabolic defects in the mesenchyme result in blastomatous malformation and abnormal differentiation of its derivatives, primarily of the adipose tissue. Ectodermal malformations are presumed to derive from the same developmental disorder which produced the mesodermal malformations (Haberland & Perou, 1970). Although there is a possibility of genetic transmission of ECCL, there are not enough cases or pedigree studies to improve upon previous speculations.

In summary, the clinical and histological findings in a child fitting most closely the syndrome of ECCL further broaden the spectrum of disease. Our patient is female while the previous three patients were male. Although previous reports had emphasized that skin lesions were unilateral, our patient had bilateral skin lesions which consisted of fibrolipomas and angiofibromas, but fulfilled all the other criteria of ECCL. The pathogenesis of ECCL remains unknown, and while no statement can be made regarding genetic transmission, it is noteworthy that our patient's mother's sister died at 2 weeks of age with 'water on the brain'.

REFERENCES

ALEXANDER, G.L. & NORMAN, R.M. (1960) *The Sturge-Weber Syndrome.* John Wright & Sons Ltd, Bristol.

CHALUB, E.G., VOLPE, J.J. & GADI, M.H. (1975) Linear naevus sebaceus syndrome associated with porencephaly and non-functioning major cerebral venous sinuses. *Neurology* **25**, 851.

FENSTEIN, R.C. & MIMS, L.C. (1962) Linear nevus sebaceous with convulsions and mental retardation. *American Journal of Diseases of Children*, **104**, 675.

FISHMAN, M., *et al.* (1978) Encephalocraniocutaneous lipomatosis. *Pediatrics*, **61**, 580–582.

GARDNER, E.W., MILLER, H.M. & LOWNEY, E.D. (1979) Folded skin associated with underlying nevus lipomatosus. *Archives of Dermatology*, **115**, 978–979.

GOLTZ, R.W., *et al.* (1970) Focal dermal hypoplasia syndrome. A review of the literature and report of two cases. *Archives of Dermatology*, **101**, 1–11.

HABERLAND, C. & PEROU, M. (1970) Encephalocraniocutaneous lipomatosis. *Archives of Neurology*, **22**, 144–155,

KOBAYASI, T., *et al.* (1973) Ultrastructure of Shagreen patch. *Acta dermato-venereologica* **53**, 275–278.

MARKS, J.G. & TOMASOVIC, J.J. (1980) Linear nevus sebaceous syndrome. *Journal of the American Academy of Dermatology*, **2**, 31–32.

RAGNE, C.J. & WOOD, M.G. (1970) Connective tissue nevus: dermatofibrosis lenticularis disseminata with osteopoikilosis. *Archives of Dermatology*, **102**, 390–396.

ROSS, C.M. (1969) Generalized folded skin with an underlying lipomatous nevus. The Michelin Tyre baby. *Archives of Dermatology*, **100**, 320–323.

SCHERER, E. (1935) Über die pialen Lipome des Gehirns. Beitrag eines Falles von ausgediechnter meningealer Lipomatose einer Grosshirnhemisphäre bei Mikrogyrie. *Zeitschrift für die gesamte Neurologie und Psychiatrie*. **145**, 45–61.

SCHORR, W.F., *et al.* (1972) The connective tissue nevus-osteopoikilosis syndrome. *Archives of Dermatology*, **106**, 208–214.

SOLOMON, L.M. & ESTERLY, N.B. (1975) Epidermal and other congenital organoid nevi. *Current Problems in Pediatrics*, **6**, 1–56.

TEMTAMY, S.A. & ROGERS, J.G.M. (1976) Macrodactyly, hemihypertrophy and connective tissue nevi: report of a new syndrome and review of the literature. *Journal of Pediatrics*, **89**, 924–927.

British Journal of Dermatology (1981) **104**, 97.

Proceedings of the 219th Meeting of the Netherlands Society for Dermatology and Venereology, Amsterdam, 26 April 1980

PRESIDENT: DR M.J.WOERDEMAN
HOST: PROFESSOR DR E.VAN DIJK

SUMMARIES OF PAPERS

Immune response due to adjuvants in penicillin preparations

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The immune response (IR) of penicillin specific antibodies after long term administration of two penicillin preparations was studied in 100 patients with syphilis. Demonstration of penicillin specific antibodies of all Ig classes was done with the ELISA. Blood was investigated before and at different times after the course. The following penicillin preparations were compared: procaine-penicillin G in oil with aluminium monostearate (Almocillin), benzathine penicillin G (Penidural) and a combination of Almocillin and Penidural. The patients could be divided into four groups according to the serology and the preparations used (Tables 1 and 2).

TABLE 1. Serology before and 1–4 months after the course

	IR	No IR
Almocillin	17 (9)	29
Almo/Penid	3 (1)	20

TABLE 2. Before and 3 weeks after the start of the course

	IR	No IR
Almocillin	6 (3)	9
Penidural	—	16

In parentheses the number of patients showing a penicillin specific IgE response.

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The differences of the IR between the groups in Tables 1 and 2 appeared to be statistically significant ($P<0{\cdot}05$). Therefore, it may be concluded that difference(s) in penicillin preparations contribute to the IR to penicillin. Much of the differences in IR can be explained by the adjuvant effects of oil and of Al monostearate in Almocillin; oil promotes the coupling of penicillin to protein (Cirstea, Suhaciu & Boros, 1975) and aluminium salts promote the synthesis of specific IgE (Pauwels *et al.*, 1979).

Moreover, penicillin blood levels are higher with Almocillin. These factors must be responsible for the humoral IR to Almocillin. Finally it should be noted that none of the patients showed any clinical allergic reaction.

REFERENCES

CIRSTEA, M., SUHACIU, G.H. & BOROS, I. (1975) Experimental studies on drug sensitization. I. Penicillin sensitization as an experimental model for drug allergy. *Revue Roumaine de Phisique*, **12**, 243.

DE HAAN, P., BOORSMA, D.M. & KALSBEEK, G.L. (1979) Penicillin hypersensitivity. Determination and classification of penicillin specific antibodies with the ELISA. *Allergy*, **34**, 111.

PAUWELS, R., BAZIN, H., PLATTEAU, B. & VAN DER STRAETEN, M. (1979) The influence of different adjuvants on the production of IgD and IgE antibodies. *Annales d' Immunologie*, **130C**, 49.

Chronic urticaria and penicillin allergy

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By means of epi-and intracutaneous tests, the possible existence of an allergy to penicillin was investigated in 252 patients with chronic urticaria (including physical urticaria). In seventy of these patients penicillin allergy could be demonstrated.

A diet free from milk and milk products was prescribed in thirty patients with a very favourable result.

Anamnestic data concerning penicillin allergy in most cases appeared to be irrelevant.

REFERENCES

DOEGLAS, H.M.G. (1975) *Chronic Urticaria.* Thesis, Groningen.

VAN KETEL, W.G. (1975) Patch testing in penicillin allergy. *Contact Dermatitis*, **1**, 253.

WARIN, R.P. & SMITH, R.J. (1976) Challenge test battery in chronic urticaria. *British Journal of Dermatology*, **94**, 401.

WARIN, R.P. & CHAMPION, R.H. (1974) *Urticaria.* W.B. Saunders, London.

Indonesian boy with lichen urticatus

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A 6-year-old, adopted Indonesian boy consulted us with lichen urticatus (strophulus) on his buttocks and back. He had a blood eosinophilia of 19%. Faeces examination in the Institute for Tropical Diseases (Amsterdam) by means of the Baerman concentration test showed thirty-seven larvae of *Strongyloïdes stercoralis (Str. st.).*

The patient was treated for 2 days with 500 mg thiabendazol (Mintezol MSD) daily. Afterwards, the faeces examination became negative and clinical symptoms subsided. Five months later there was a relapse of the skin eruption and faeces examination revealed three larvae of *Str. st.* again with a blood eosinophilia of 14%. *Str. st.* is an ovoviviparous worm. Only the larvae can be found in fresh faeces. The examination however is reliable only in specialized laboratories. In general infestation with *Str. st.* gives rise to minor complaints: lichen urticatus and a mild diarrhoea. However, if the resistance of the host is impaired, for example by treatment with corticosteroids or cytostatics, serious symptoms can result.

REFERENCES

ROOK, A., WILKINSON, D.S. & EBLING, F.J.G. (1979) *Textbook of Dermatology*, 3rd ed. Blackwell Scientific Publications, Oxford.

JOYEUX, CH. (1944) *Précis de Medicine Coloniale.* Masson et Cie, Paris.

ZUIDEMA, P.J. (1976) Diagnostiek en behandeling van nematodeninfecties van het darmkanaal bij migranten uit Suriname. *Nederlands Tijdschrift voor Geneeskunde*, **120**, 1849; 1855.

Tuberous sclerosis. An investigation into the ratio of sporadic versus familial cases.

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According to the literature, the mutation rate of tuberous sclerosis (T.S.) is approximately 75%. As severely affected patients are unlikely to have children, a high percentage of spontaneous mutations is to be expected. The incidence of T.S. in the Netherlands is estimated to be 1:30,000. At a mutation rate of 75% this means one new mutation in 40,000 births. This frequency is in accordance with the literature but is considerably higher than in other dominant diseases.

The high percentage in the literature is based on family investigations performed before the significance of the hypomelanotic spots was appreciated and before cranial computed tomography (CCT) was available.

We examined ninety-five parents of forty-eight unrelated affected children dermatologically, ophthalmologically and neurologically. In all parents an EEG, X-ray of the skull and CCT were made. Electronmicroscopy was done of clinically doubtful white spots. Genealogical investigation was undertaken in all families.

Thirteen children were found to have one affected parent. The parents of twelve children were considered unaffected; twenty-three children had one parent with one or two questionable signs. Twelve of the thirteen affected parents had skin symptoms: hypomelanotic spots were present in all and in six these spots were combined with other dermatological manifestations. All thirteen had at least one non-dermatological symptom. CCT was positive in seven and probably positive in two more parents.

In twenty-three cases we are still in doubt. These had mostly hypomelanotic spots, sometimes combined with probable ophthalmological symptoms. Electronmicroscopy of these spots is being done.

No relationship was found between the forty-eight families in five generations.

Our preliminary results are in accordance with the literature. Only thirteen of 48 children had undoubtedly inherited T.S. However the mutation rate may eventually prove to be lower depending on the results of the electron microscopic investigation in the twenty-three undecided cases. In our series of thirteen affected parents, the hypomelanotic spots were the most frequent sign of T.S.; a positive CCT was the next most frequent one.

REFERENCES

GUNTHER, M. & PENROSE, L.S. (1935) The genetics of epiloia. *Journal of Genetics*, 31, 413.

NEVIN, N.C. & PEARCE, W.G. (1968) Diagnostic and genetical aspects of tuberous sclerosis. *Journal of Medical Genetics* 5, 273.

ZAREMBA, J. (1968) Tuberous sclerosis: clinical and genetical investigation. *Journal of Mental Deficiency Research*, 12, 63.

FITZPATRICK, T.B., SZABO, G., HORI, Y., SIMONE, A.A., REED, W.B. & GREENBERG, M.H. (1968) White leaf-shaped macules. Earliest visible sign of tuberous sclerosis. *Archives of Dermatology*, 98, 1.

Tuberous sclerosis. Ultrastructure study of white spots in patients and parents

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White spots may be the first symptom of tuberous sclerosis (T.S.). In otherwise healthy parents of children with T.S. similar spots are often found. Biopsies were taken from hypomelanotic and adjacent normal skin of three patients and three parents for ultrastructural study of the pigment system. A comparison between quantity, melanization and size of melanosomes was made. The mean size of melanosomes was measured according to the method used by Jimbow *et al.* (1975).

Results. The hypomelanotic skin of the patients showed decrease in quantity, melanization and size of melanosomes. In two parents there was a slight decrease in quantity and melanization but a marked decrease in size of the melanosomes. In the skin of the third parent no difference was found.

Conclusion. Ultrastructural study of the white spots in families with T.S. may prove to be a helpful method in genetic counselling.

REFERENCES

JIMBOW, K., FITZPATRICK, T.B., SZABO, G. & HORI, Y. (1975) Congenital circumscribed hypomelanosis: a characterization based on electron microscopic study of tuberous sclerosis, nevus depigmentosus and piebaldism. *The Journal of Investigative Dermatology*, 64, 50.

Contact allergy to clioquinol and neomycin

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The persistence of positive patch test reactions to clioquinol and neomycin was investigated.

Four to six years after the first positive reaction to clioquinol, all ten patients showed definite positive patch test reactions to this compound. From a group of ten patients allergic to neomycin, eight showed positive patch test reactions to neomycin after 4–6 years, while four of these eight patients also appeared to be hypersensitive to gentamycin.

The hypersensitivity persisted, in spite of the fact that all patients had avoided contact with clioquinol or neomycin.

Retarded (5–7 day) skin reactions to recall antigens

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When testing with recall antigens, sometimes a prolonged skin reaction with a maximum on the 5th–7th day is found. This type of reaction was described earlier in connection with trichophytids and erythema annulare centrifugum (EAC) and has been named 'retarded reaction' (RR) (Wood & Cruickshank, 1962).

In our investigation 100 normals, forty-two patients with *Trichophyton rubrum* infection, eleven patients with EAC, eight patients with lymphocytic infiltration of Jessner type and eight patients with trichophytids were tested intracutaneously with recall antigens. RR was found in ten of eleven patients with EAC, six of eight patients with lymphocytic infiltrations and six of eight with id-reactions. The normals and the patients with *Trichophyton rubrum* infection showed a RR in only 5%. Only one RR was found in each patient (except one), suggesting specificity. Trichophytin (13), PPD (11) and candidin (3) were most often found. There is a strong correlation between EAC, clinical history and RR to PPD, and between mycotic infections, id-reactions and RR to trichophytin.

These data suggest that in certain diseases a specific retarded skin reaction can be found to a relevant antigen. The mechanism of this reaction is still obscure. Therapeutic measures (e.g. INH or griseofulvin) were encouraging in EAC but less so in id-reactions.

REFERENCES

WOOD, S.R. & CRUICKSHANK, C.N.D. (1962) The relation between trichophytin sensitivity and fungal infection. *British Journal of Dermatology*, **74**, 329.

Treatment of chronic palmo-plantar dermatoses with PUVA

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Published in *Zeitschrift für Hautkrankheiten* (1980), **55**, 523.

Patterns of immunofluorescence of the skin in healthy individuals

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To be published elsewhere.

Extension of the molluscum contagiosum lesion

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To be published elsewhere.

Correlation between *in vitro*- and *in vivo*-tests in penicillin hypersensitivity

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To be published elsewhere.

Immunologic aspects of mycotic infections

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To be published elsewhere.

Epidermolysis bullosa acquisita: an (immune) electronmicroscopic and immunohistochemical study

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Published in *British Journal of Dermatology* (1980) **102**, 383.

CASE REPORTS

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Herpetiform pemphigus

W.H.H.W.van den Berg

H.H., female, aged 83 years.

History. Since 1974 the patient has experienced periods of blistering and severe itching, alternating with periods without any complaint. In 1979, a severe exacerbation occurred, with bullous lesions on the oral mucosa. No medicaments are used.

Examination. The trunk presents some dozens of red annular lesions, with a diameter of 3–8 cm. The border is slightly raised and shows numerous pinpoint to lenticular vesicles and bullae (Fig. 1).

On the buttocks, lower arms, dorsa of the hands and on the legs there are many disseminated vesicles, bullae on an erythematous base and also pustules. The Nikolsky sign is negative. The oral mucosa shows a small number of bullae and erosions.

Histology. (1974) The epidermis shows acanthosis, considerable spongiosis and exocytosis with many eosinophils. There is some evidence of partial acantholysis. The upper dermis shows oedema and widened blood vessels with a moderate perivascular mononuclear and granulocytic infiltrate. Numerous granulocytic cells with many eosinophils are scattered throughout the dermis. Diagnosis: eosinophilic spongiosis (1979). Essentially the same findings are observed as in 1974. However suprabasal acantholysis with small blisters is more prominent (Fig. 2). Conclusion: This picture resembles pemphigus vulgaris.

Cytology. Occasional Tzanck cells and many polymorphonuclear leukocytes with numerous eosinophils are seen.

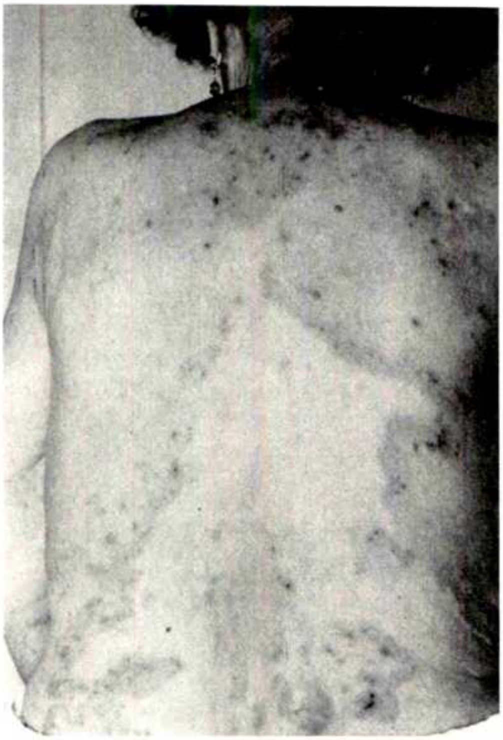

FIGURE 1. Gyrate urticarial lesions with some blistering and erosions on the back.

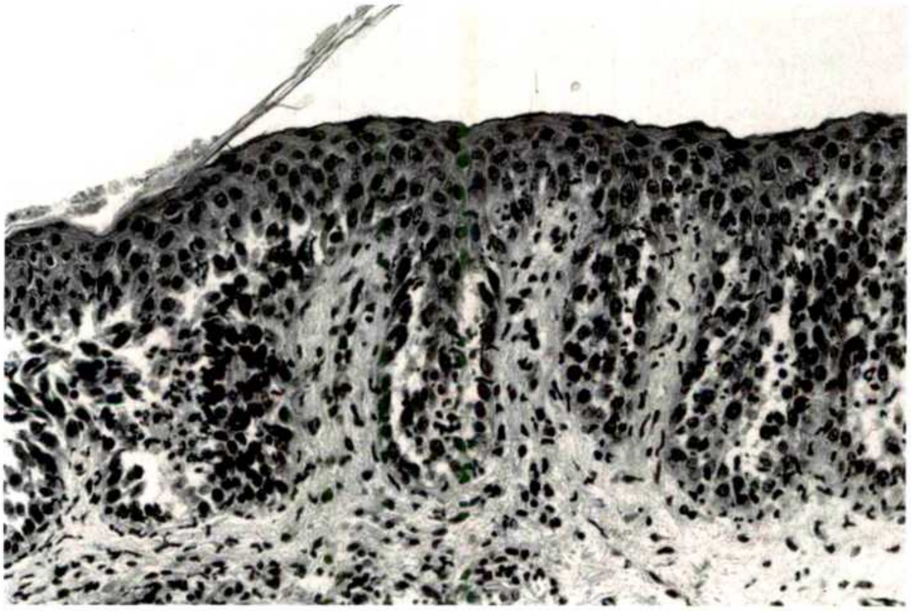

FIGURE 2. Eosinophilic spongiosis and suprabasal acantholysis.

Immunofluorescence (IF). Direct IF: Only in the diseased skin is there a granular staining of the epidermis with anti-IgG, whereas intercellular staining is seen with anti-C3/4. Granular IgG deposits are present along the basement membrane and between the basal cells. Indirect IF: Pemphigus-type antibodies are detectable 1:16 (1979).

Investigations. Eosinophilia of the peripheral blood, slight anaemia, ESR 65 mm/1 h.

Treatment. In 1974, dapsone was given without any improvement; the lesions subsided with oral betamethasone 17-valerate. In 1979 the treatment consisted of 50 mg prednisone daily after which the lesions disappeared.

Comment. The macroscopic and microscopic findings and the course were not consistent with the diagnosis pemphigus vulgaris. They have been described as herpetiform pemphigus. It is still questionable whether herpetiform pemphigus is a distinct entity, as the condition may change into pemphigus foliaceus (Sneddon & Church, 1967).

REFERENCES

EMMERSON, R.W. & WILSON JONES, E. (1968) Eosinophilic spongiosis in pemphigus. A report of an unusual histological change in pemphigus. *Archives of Dermatology*, 97, 252.

HAENSCH, R. (1979) Pemphigus herpetiformis. *Hautarzt*, 30, 418.

JABLONSKA, S., CHORZELSKI, T.P., BEUTNER, E.H. & CHORZELSKA, J. (1975) Herpetiform pemphigus, a variable pattern of pemphigus. *International Journal of Dermatology*, 14, 353.

KNIGHT, A.G., BLACK, M.M. & DELANEY, T.J. (1976) Eosinophilic spongiosis. *Clinical and Experimental Dermatology*, 1, 141.

SNEDDON, I. & CHURCH, R. (1967) Pemphigus foliaceus presenting as dermatitis herpetiformis. *Acta dermato-venereologica*, 47, 440.

Zosteriform lentiginous naevus

J.C. VAN DER HORST AND H.J. DIRKSEN

F.d.V., female, aged 23 years.

History. In the first year of life a light brown patch with small dark brown macules appeared on the left arm. The area gradually extended with a relatively rapid growing phase during puberty. Hyperplasia of the gingiva has been present since birth. At the age of 16 years focal epilepsy became manifest. There is no family history of similar conditions.

Examination. The left arm presents an extensive band-like light-brown pigmented lesion with numerous small brown or dark-brown spots, some of which are slightly raised. Also some small flesh coloured soft tumours are seen (Fig. 3). The oral mucosa shows bilateral hyperplasia of the gingiva.

Histology. (a) Dark-brown slightly raised lesion: in and beneath the basal layers of the epidermis, naevus cell nests are present, with increase of melanin. The dermis shows disseminated melanophages. (b) Flesh-coloured tumour: increase of collagen and a number of cells with spindle shaped and oval nuclei, some of them double nuclei. Van Gieson-elastin stain: elastin fibres are absent in mid- and upper-dermis. Diagnosis: neural naevus.

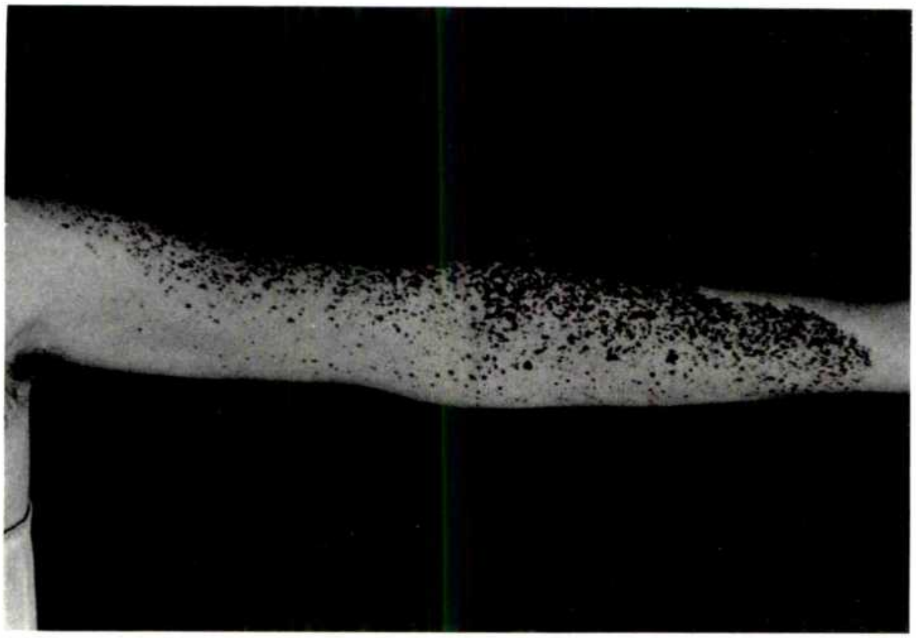

FIGURE 3. Zosteriform pale-brown area on the left arm showing many dark brown spots.

Electronmicroscopy. (Dark-brown slightly raised lesion and light-brown area: both biopsies presented similar findings.) Melanin is present in the stratum Malpighii and especially in the basal layer as melanosomes, melanosome-complexes and round giant melanosomes. The latter are observed in the keratinocytes, melanocytes and naevus cells. Dermis: numerous macrophages containing many melanosome complexes, some of these also containing round giant melanosomes. No difference is observed between the darker and the lighter brown lesion.

Treatment. Plastic surgery.

Comment. This patient presents the features of zosteriform lentiginous naevus as has been described by Matsudo *et al.* (1973). Electronmicroscopy, not done before in this type of lesion, revealed round giant melanosomes as have also been found in naevus spilus and von Recklinghausen's disease. Although malignant degeneration in this condition has not been described, excision was performed for cosmetic reasons and because of the presence of junctional activity.

REFERENCES

JIMBOW, K., SZABO, G. & FITZPATRICK, T.B. (1973) Ultrastructure of giant pigment granules (macromelanosomes) in the cutaneous pigmented macules of neurofibromatosis. *Journal of Investigative Dermatology*, **61**, 300.

MATSUDO, H., REED, W.B., HOMME, D., BARTOK, W. & HOROWITZ, R. (1973) Zosteriform lentiginous nevus. *Archives of Dermatology*, **107**, 902.

PORT, M., COURNIOTES, J. & PODWAL, M. (1978) Zosteriform lentiginous naevus with ipsilateral rigid cavus foot. *British Journal of Dermatology*, **98**, 693.

Pigmented naevi following Stevens-Johnson syndrome

J.C. van der Horst and J. van Baak

R.v.V., female, aged 31 years.

History. In 1975 the patient had Stevens-Johnson syndrome with bullous lesions on the feet and in the region of the nails. Afterwards changes appeared in the finger and toenails. In 1977, small brown macules became present on the right sole and were gradually progressive.

Examination. On the sole, extending to the sides and the dorsum of the right foot, there are some dozens of brown macules 2–6 mm in diameter (Fig. 4). The finger and toenails are dystrophic.

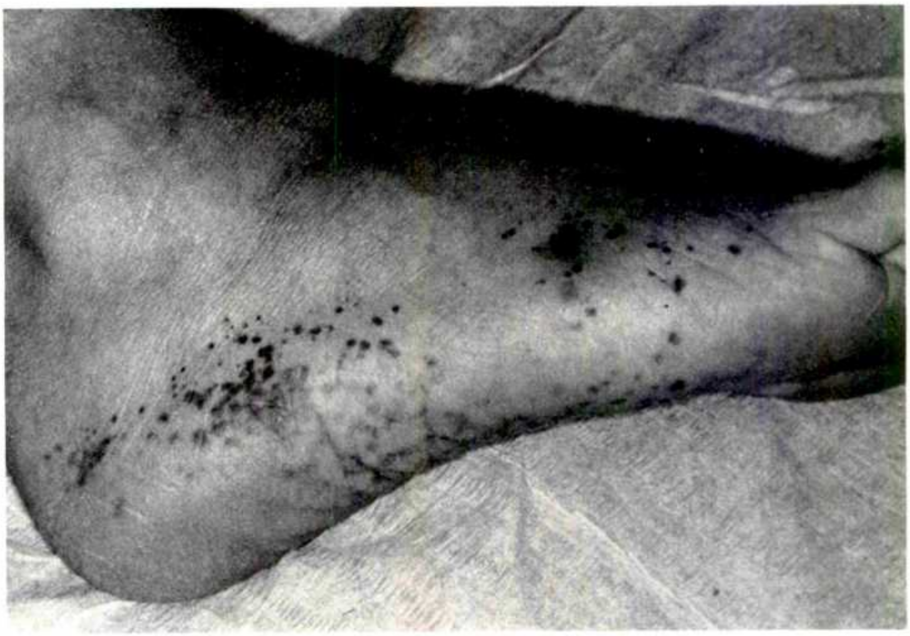

FIGURE 4. Pigmented naevi on the right foot.

Histology. Some naevus cell nests in the basal layers of the epidermis are seen; there is increase of melanin pigment locally also in the horny layer. The dermis contains several melanophages.

Laboratory investigations. No abnormalities; mycological examination of the nails negative.

Treatment. None.

REFERENCES

Garz, G. & Tsambaos, D. (1978) Eruptive nevocytic nevi after Lyell's syndrome. *Archives of Dermatology*, **114**, 1400.

Kopf, A.W., Grupper, C., Baer, R.L. & Mitchell, J.C. (1977) Eruptive nevocytic nevi after severe bullous disease. *Archives of Dermatology*, **113**, 1080.

Saltani, K., Bernstein, J.E. & Lorincz, A.L. (1979) Eruptive nevocytic nevi following erythema multiforme. *Journal of the American Academy of Dermatology*, **1**, 503.

Cowden's disease

TH.M.STARINK, P.K.S.CIRKEL AND B.KOOPMANS-VAN DORP

C.H., female, aged 39 years.

History. The eruption started in childhood. The past medical history reveals palpable enlargement of the thyroid, medical treatment for a questionable hyperthyroidism in 1973 and bilateral mastectomy for fibrocystic disease with malignant degeneration in 1974. Preliminary examination of the large Dutch family of this patient revealed three other cases.

Examination. On the face, especially on the eyelids and on and around the ears, there were numerous pinpoint skin coloured papules. The distal part of the extremities showed many pea-sized keratotic and verrucous lesions. Punctate, translucent keratotic papules with a central dell were present on the palms and soles. The oral mucosa showed small papules; larger areas with a cobblestone appearance were present on the gingivae.

Histology. Facial papule: no abnormalities. Keratosis on the lower arm: circumscribed depression with hyperkeratosis, hypergranulosis and acanthosis of the epidermis with convergence of the peripheral rete ridges. The upper dermis showed dilated capillaries and a mild mononuclear cell infiltrate. Keratosis on the lower leg: in connection with hair follicles two invaginations were present, filled with keratin and covered by an acanthotic wall. Plantar keratosis: beneath and in continuity with the epidermis there was a globular tumour of squamous cells not containing glycogen and with a central keratin mass. Several transverse sections of sweat ducts were present. There was arborization of rete ridges at the periphery of the tumour. A deeper section showed a connection of the central keratin mass with the acanthotic epidermis (Fig. 5).

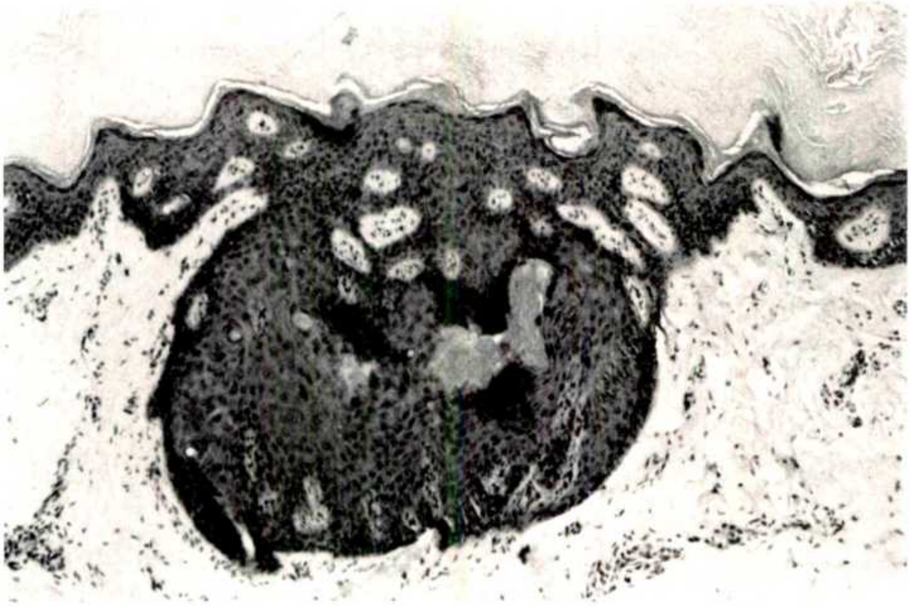

FIGURE 5. Plantar keratosis showing a globular tumour.

Investigations. Routine blood examination and thyroid function tests: no abnormalities. Gastrointestinal examination: in preparation.

Comment. The patient presents the typical clinical features of Cowden's disease. We could not demonstrate facial tricholemmoma. The convergence of rete ridges in the other biopsies was remarkable although further evidence for viral warts was absent. The history of the plantar keratosis suggests an unusual type of sweat gland duct hamartoma not described previously.

REFERENCES

BROWNSTEIN, M.H., MEHREGAN, A.H., BIKOWSKI, J.B., LUPULESCU, A. & PATTERSON, J.C. (1979) The dermatopathology of Cowden's syndrome. *British Journal of Dermatology*, **100**, 667.

LAUGIER, P., KUFFER, R., OLMOS, L., HUNZIKER, N., ROUGIER, M. & FIORE-DONNO, G. (1979) Maladie de Cowden. A propos de 8 cas familiaux. *Annales de Dermatologie et de Vénéréologie*, **106**, 453.

WADE, T.R. (1979) Cowden's disease. *Cutis (N.Y.)*, **24**, 537.

Congenital hypertrichosis lanuginosa

A.J.LANDWEHR

M.K., male, aged 4 years.

History. The hypertrichosis has been present since birth; in infancy the hair gradually lengthened. The patient is healthy. The family history is negative.

Examination. On the upper and lower back there is a somewhat pear-shaped area with fine silky hairs up to 10 cm long (Fig. 6). In this area the skin is pink in colour with a perifollicular yellow hue. Long eyelashes and thick eyebrows are notably absent. The upper part of the ears are somewhat pointed, the lobes of the ear being hypoplastic. On the nape of the neck, on the face and on the breast are some dozens of nummular areas with a similar appearance. Besides epicanthus and hypertelorism no other abnormalities can be found.

Histology. No abnormalities.

Treatment. Shaving.

Comment. The patient shows a number of unusual findings for this rare autosomal dominant disease: a relatively limited extent of hypertrichosis could not be found in the literature. It is not clear whether there is a relationship with the abnormal shape of the ears and the hypertelorism since these abnormalities have not been reported as associated findings in this disease.

REFERENCES

BEIGHTON, P. (1970) Congenital hypertrichosis lanuginosa. *Archives of Dermatology*, **101**, 669.

FELGENHAUER, W.R. (1969) Hypertrichosis lanuginosa universalis. *Journal Génétique humaine*, **17**, 1.

NOWAKOWSKI, T.K. & SCHOLZ, A. (1977) Geschichte der Dermatologie: Das Schicksal behaarter Menschen im Wandel der Geschichte. *Der Hautarzt*, **28**, 593.

SUSKIND, R. & ESTERLY, N.B. (1971) Congenital hypertrichosis universalis. *Birth Defects: Original Articles Series*, **VII**, 103.

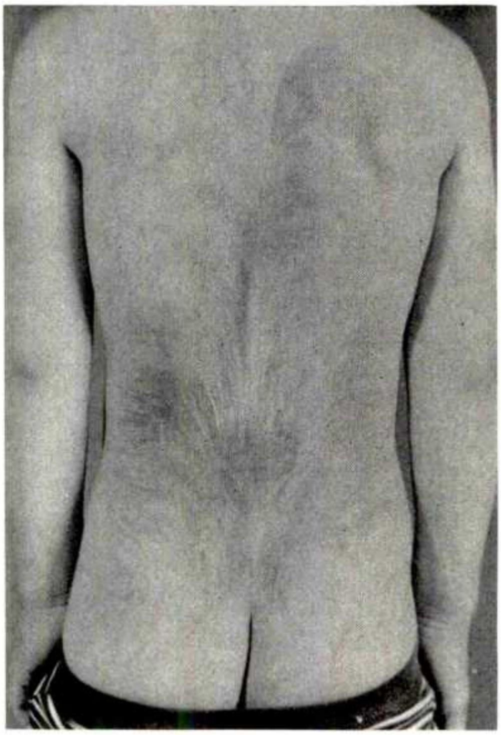

FIGURE 6. Serrated pear shaped area on the back with fine silky hairs.

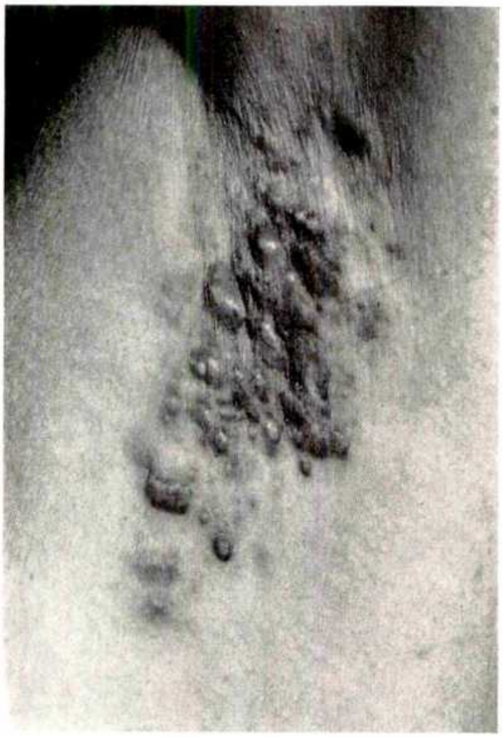

FIGURE 7. Cystic lesions in the left axilla.

Xanthomatosis cutis in a patient with hyperlipoproteinaemia, Frederickson type II B, with unusual localization and cystic lesions

E.FOLKERS, J.A.SCHOUTEN AND A.W.THÖNE

C.J.M.S., female, aged 57 years.

History. For 3 years, lesions have been present around the eyes and in the axillae. One sister also appears to have hyperlipoproteinaemia, Frederickson type II B.

Examination. Both axillae show a brown-red eruption with yellow-coloured cysts containing white-yellow semiviscous material (Fig. 7). Around the eyes cysts are also present. Red-yellow papular xanthomas are seen on the upper limbs and the hands.

Investigations. Cholesterol, 7·9 mmol/l, triglycerides, 3 mmol/l; other investigations normal.

Histology. Necrotic foci are present in the dermis surrounded by granulomatous inflammation. In the upper dermis there are numerous xanthoma cells containing lipids (Sudan-black stain).

Treatment. Diet and cholestyramine (Questran) therapy resulted in disappearance of all lesions.

Comment. Localization of xanthomas in the flexures in patients with hyperlipoproteinaemia, Frederickson type II B, is rare. The formation of cysts of semiviscous lipid has seldom been reported.

REFERENCES

UNDEUTSCH, D. (1976) *Vetstofwisselingsstoornissen*, Hoofdstuk 14. Editiones Roche, Basel.

BERNSTEIN, S.S., WILLIAMS, H.H., HUMMEL, F.C., SHEPHERD, M.L. & ERICKSON, B.N. (1939) Metabolic observations on a child with essential hyperlipemia. *The Journal of Pediatrics*, 14, 570.

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Book Reviews

Clinical Practice in Sexually Transmissible Diseases. By D.H.H.ROBERTSON, A.MCMILLAN and H.YOUNG (1980) Tunbridge Wells: Pitman Medical Limited. Pp. 458. Price £27.00.

Scotland has given us many good things and it is a pleasure to welcome amongst them this new textbook so neatly entitled 'Clinical Practice in Sexually Transmissible Diseases'. The care that has gone into choosing this title is evident on every page of the book.

The clinical aspects of diagnosis and treatment of sexually transmitted diseases, and their detailed management, are accomplished brilliantly in 425 readable pages. There is an excellent introductory chapter which details with the epidemiology and control of these disorders and reviews many of the medico-legal aspects of the problems surrounding them.

Syphilis and gonorrhoea are well dealt with, with the section on treatment and follow-up of the former being especially practical and detailed. The treatment of gonorrhoea is comprehensive, clear and up to date.

Non-gonococcal urethritis, a subject which many authors often find a confusing one to write about, is dealt with in a very clear and readable manner, though I was a little disappointed to see the authors suggest a low priority in the tracing of female contacts of men with non-specific urethritis—surely these women are at risk of developing pelvic inflammatory disease themselves, of infecting their babies with ophthalmia, and there is always the possibility of re-infecting their sexual partners.

Excellent accounts are given of genital herpes and sexually transmitted viral hepatitis. Sensible advice about the management of delivery in mothers with genital herpes is given, whilst the whole question of carcinoma of the cervix is carefully considered in a separate chapter.

Some further idea of the comprehensive nature of the book may be obtained by the presence of chapters on 'frequency and dysuria in urinary tract infections' and 'diseases due to intestinal organisms in homosexual males'.

Some very good colour photographs are included, though unfortunately all are bound together in a single section.

I recommend this book to anyone with an interest in this group of illnesses. It will be of use to the serious student or the practising doctor (whatever his professional status) and neither can fail to read it without interest and profit. At £27, however, the book is rather on the expensive side even for 1980.

J.K.OATES

Venereology in Practice. The Sexually Committed Diseases. By NIELS HJORTH, HENNING SCHMIDT and HOWARD I.MAIBACH (1980) London: YB Medical Publishers Limited. Pp. 108, 40 col. illus. Price £9.75.

This small book declares in its introduction that the sexually transmitted diseases are 'reviewed as thoroughly as their present prevalences warrant' and that the text is intended to provide the amount of knowledge in the management of sexually transmitted diseases expected of a practising physician. In a volume of fifty-nine pages this is quite a task. One of the problems of the small precis-type volume is that errors tend to stand out far more clearly than in a larger publication, and this is certainly true here.

In general, however, the authors succeed in their task of wasting few words yet giving reasonably accurate accounts of most of the sexually transmitted diseases, their diagnosis, management and modern treatment. Balance, however, is less well held and perhaps the dermatological background of the authors is responsible for the fact that Reiter's disease is given thirty-two lines against forty-five for pediculosis and sixty-two for scabies. The major diseases of gonorrhoea and syphilis are reasonably well dealt with and the text is one of the few that I have seen which points out that gonococcal salpingitis can occasionally be unilateral and which also stresses the fact that up to 30% of men with gonococcal urethritis may have no symptoms. On the other hand, is it really true that only 20% of women are sterile as the result of gonococcal pelvic inflammatory disease, and gonococcal stricture *is* an entity and is not due to irrigation with silver nitrate as is stated in the text. And what is one to make of the odd remark that 'gonorrhoea is rarely the cause of an enlarged prostate'? It is in my view a pity that they recommend methylene blue stain for the microscopic diagnosis of gonorrhoea as this method is only reasonably accurate in the case of male urethral gonorrhoea.

Pages on syphilis deal well with most aspects of early disease, diagnosis, modern serological tests and treatment. Though cardiovascular syphilis is nowadays very rare, I'm sure few physicians in this country would agree with one of the schedules quoted for its treatment, namely benzathine penicillin 2·4 g intramuscularly weekly for a total of three doses.

Colour photographs are amongst the best I have seen in a text book and all are very skilfully chosen. With a little more attention to detail and especially to translation this will be an excellent short volume which, within the limitations of size, can be recommended.

J.K.OATES

A Colour Atlas of Hand Conditions. By BRUCE W.CONOLLY (1980) London: Wolfe Medical Publications. Pp. 344, 826 colour and 228 b/w photographs. Price £35.00.

The importance of the colour atlas has been recognized since Alexander Balmanno Squire published his atlas on Diseases of the Skin in 1878. The English edition of Jacobi's Portfolio of Dermochromes arrived later in the early 1900s. Colour atlasses have now extended their range to nearly every branch of medicine. 'A Colour Atlas of Hand Conditions' is an impressive addition to the imposing list produced by Wolfe Medical Publications. This atlas is not for the intending or practising dermatologist. In contrast to many of the other illustrations, those featuring dermatological conditions are few, of poor quality, and require the dermatologist to look at the caption, as in most cases it is impossible to make a diagnosis from the photograph.

In the section labelled 'miscellaneous infections', there is a misplaced dusky and succulent solitary lesion of granuloma annulare with the words 'it can ulcerate'. On the next page another plate shows a hand with dystrophic fingernails, exhibiting a glazed circumscribed lesion encircling the glans penis. 'Fungal infection of nails and glans penis' reads the caption; neat but of doubtful veracity.

Elsewhere the surgeon author is on more certain ground. The section on anatomy and function of the normal hand is especially clear and informative; those on congenital disorders, injuries and vascular and painful disorders are profusely well illustrated with excellent colour photographs, black and white diagrams and tables.

These and the other surgically orientated sections provide succinct and digestible information for the Fellowship candidate and casualty officer. This atlas is a good buy, if an expensive one, for any accident and emergency department library.

P. HALL SMITH

Collagenase in Normal and Pathological Connective Tissues. By DAVID E.WOOLLEY and JOHN M.EVANSON (1980) Chichester: John Wiley & Sons Limited. Pp. 292, B/W illustrations. Price £16.00.

Mammalian collagenases are a varied enzyme family produced by cells as diverse as leukocytes, macrophages, fibroblasts and probably epithelial cells. They share the ability to cleave animal collagens into 3/4 and 1/4 N and C terminal fragments which can then be further digested by other less specific proteinases. Bacterial collagenases on the other hand digest animal collagens to very small peptide fragments. Animal collagenases probably help to regulate a variety of physiological and pathological processes including wound healing, keloid scar formation, peridontal disease, osteoporosis, rheumatoid arthritis and postpartum uterine regression. Specific basement membrane collagenases may determine the degree of malignancy (by metastases) of certain epithelial or glandular cancers. Altered collagenase activity or structure is possibly relevant to several dermatological diseases including scleroderma, various dermal atrophies and acquired subepidermal bullae.

This book contains contributions from twenty-two prominent collagen and collagenase-ologists from the USA, Europe and the United Kingdom who attended a 1978 Symposium in Manchester. It comprehensively reviews the collagenase field and is a valuable introductory cookbook for those researchers new to the field as well as containing much of great interest to established workers in the field. It is well produced and printed and will be a valuable addition to the library shelves of academic departments of dermatology, rheumatology, immunology, metabolic bone disease, dentistry, pathology, biochemistry and cell biology.

F.M.POPE

Dermatologia. By J.A.ESTEVES, A.P.BAPTISTA and F.G.RODRIGO (1980) (Text in Portuguese) Lisbon: Calouste Gulbenkian Foundation. Pp. 1485, 421 figures.

This substantial new text book has many virtues. The authors have achieved a very satisfactory balance between basic science and clinical practise, and have given more detailed information on the practical aspects of treatment than most books provide. The illustrations, the majority of which are excellent, include many conditions now rare in Britain.

Many English speaking dermatologists will find the several pages of contact dermatitis statistics of particular interest. Not even the endearing complexities of the Portuguese language can conceal the identity of Crómio or Tiuram-mix!

ARTHUR ROOK

British Journal of Dermatology (1981) **104,** 115.

News and Notices

B.A.D. Travelling Fellowships

Applications are invited from senior registrars/lecturers in dermatology as well as from recently appointed consultants and senior lecturers for the British Association of Dermatologists Travelling Fellowships. The Fellowships will be awarded for a period of 3 weeks and arrangements will be made for the Fellows to visit various centres in the United States/Canada and to attend one of the National Meetings in Dermatology. The appropriate travel and subsistence expenses of the Fellows would be borne by the B.A.D. Further information and application forms can be obtained from Dr G.M.Levene, Honorary Secretary, British Association of Dermatologists, 7 John Street, London WC1N 2ES.

Completed applications should be returned by 1 February 1981.

Cutaneous Lymphoma. The 6th Clinically Oriented Symposium of the E.S.D.R.

The 6th Clinically Oriented Symposium of the European Society for Dermatological Research will be held in Kiel, February 13–14 1981, organized by The Department of Dermatology of the University of Kiel. The subject is Cutaneous Lymphoma.

The preliminary Programme includes:

(1) Basic aspects: origin and function of Langerhans cells; structure and function of stroma cells in lymphoid tissue; histogenesis of T and B lymphocytes; development and significance of germinal centres; lymphocyte hybridomas; functional properties of malignant lymphomas; leukaemia *vs* lymphoma.

(2) Clinical pathology: Langerhans cells in disease; malignant histiocytosis; Merkel cell neoplasms; T cell lymphomas; B cell lymphomas.

(3) Current cooperative studies and trials in cutaneous lymphoma.

(4) Methodological seminar.

(5) Free communications.

The Guest Faculty includes international experts in the field of lymphoma and immunology.

Further information may be obtained from: E.Christophers, M.Goos, Department of Dermatology, Schittenhelmstrasse 7, D-2300 Kiel, Germany.

University of California, San Francisco

A dermatology consultants' course will be held at The Ahwahnee Hotel, Yosemite, California, 8–11 March 1981.

Further information may be obtained from: Extended Programmes in Medical Education, School of Medicine, Room 569-U, San Francisco, California 94143, USA.

International Society of Tropical Dermatology

The *Scandinavian part* of the meeting will be held in Copenhagen/Lund on 9th–11th April 1981.

There will be several short communications from the staff of the dermatological departments of Rigshospitalet, Gentofte Hospital and Laserettet Lund; also speakers outside Scandinavia have been invited, mainly from the U.S.

The programme from Rigshospitalet covers mainly connective diseases, especially scleroderma and investigations regarding immunology and histopathology. The programme from Lund and Gentofte is focused mainly on occupational dermatology and melanoma.

The chairman of the meeting is Gunnar Lomholt, M.D., Department of Dermatology, Laserettet, S-251 87 Helsingborg, Sweden.

First International Symposium on Minimal Invasive Cancer

The First International Symposium on Minimal Invasive Cancer, organized by the University Clinic of Obstetrics and Gynaecology, will be held in Graz, Austria, 1st–4th July 1981. The Congress language will be English.

The main topics will be: cervix, vulva, endometrium, breast, oesophagus, stomach, large intestine, lung, thyroid, urinary bladder, prostate, larynx, oral cavity, skin, other organs.

Further information may be obtained from: Interconvention, PO Box 105, A-1014 Vienna, Austria.

3rd Annual Dermatopathology Colloquium

This meeting will take place at Guy's Hospital, London, SE1 on July 11th 1981 immediately preceding the International Dermatopathology Symposium. Further information regarding applications for attendance and details relating to submission of abstracts may be obtained from Dr D.M.MacDonald, Department of Dermatology, Guy's Hospital, London, SE1 9RT.

Advanced Seminars in Dermatology

16–20 September 1981, Carmel, Monterey, California

The 1981 Advanced Seminars held at Carmel, Monterey, California, 16–20 September 1981 will, in addition to the regular programme, offer category I and IAE credit for two special seminars: Cutaneous Viral Disease and Dermatopathology and Immunopathology. Special guest speakers include Drs S. Jablonska and A.Ackerman.

For a programme and registration information, please write to: Advanced Seminars in Dermatology, c/o University of California Medical School, Dermatology Department, San Francisco, CA 94143, USA.

APPOINTMENT

Lancaster and Kendal

Dr Philip Harrison has been appointed Consultant Dermatologist based at Lancaster and Kendal.

Graduating from Edinburgh in 1973 and training in general medicine at Aberdeen, dermatology then took him successively to Newcastle, Leeds and Glasgow. His interests include histopathology, cutaneous surgery—and mountains.

Another Munksgaard Journal

Contact Dermatitis

Environmental and Occupational Dermatitis

Editor
C. D. CALNAN, M.D., London

Contact Dermatitis is designed primarily as a journal for clinicians who are interested in various aspects of environmental dermatitis. This includes both allergic and irritant (toxic) types of contact dermatitis, occupational (industrial) dermatitis and consumers' dermatitis from such products as cosmetics and toiletries.

The journal aims at promoting and maintaining communication among dermatologists, industrial physicians, allergists and clinical immunologists, as well as chemists and research workers involved in industry and the production of consumer goods.

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NOTICE TO CONTRIBUTORS

The *British Journal of Dermatology* publishes original articles on all aspects of the normal biology, and of the pathology of the skin. Originally the Journal, founded in 1888, was devoted almost exclusively to the interests of the dermatologist in clinical practice. However, the rapid development, during the past twenty years, of research on the physiology and experimental pathology of the skin has been reflected in the contents of the Journal, which now provides a vehicle for the publication of both experimental and clinical research and serves equally the laboratory worker and the clinician. To bridge the gap between laboratory and clinic the Journal publishes signed editorials reviewing, primarily for the benefit of the clinician, advances in laboratory research, and also reviews of recent advances in such aspects of dermatology as contact dermatitis, therapeutics and drug reactions. Other regular features include book reviews, correspondence and society proceedings. The Journal is the official organ of the British Association of Dermatologists and also of the Netherlands Association for Dermatology and Venereology, but it attracts contributions from all countries in which sound research is carried out, and its circulation is equally international.

Papers accepted become the copyright of the Journal. This journal is covered by *Current Contents*.

Manuscripts should be sent to the Editor, Dr R.H.Champion, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ. They should be typewritten on one side of the paper only, with a wide margin, be double spaced, and bear the title of the paper, name and address of each author, together with the name of the hospital laboratory or institution where the work has been carried out. The name and full postal address of the author who will be responsible for reading the proofs should be given on the first page. If, in addition to the original typewritten copy, a duplicate copy, complete with figures, tables and references, is submitted, this will frequently speed publication. The author should keep a carbon copy of his manuscript. The Editorial Board reserves the right to make literary corrections.

Abbreviations and units. Full names with uncommon abbreviations must be given with the first mention: new abbreviations should be coined only for unwieldy names and should not be used at all unless the names occur frequently. In the title unusual abbreviations should be identified, in the introduction and discussion they should be used sparingly. Abbreviations of units should conform with those shown below.

gram(s)	g	minute(s)	min	molar	mol/l
kilogram(s)	kg	centimetre(s)	cm	millilitre(s)	ml
milligram(s) (10^{-3} g)	mg	second(s)	s	gravitational acceleration	*g*
microgram(s) (10^{-6} g)	μg	cubic millimetre(s)	mm^3	micrometre(s)	μm
nanogram(s) (10^{-9} g)	ng	millimetre(s)	mm	per cent	%
picogram(s) (10^{-12} g)	pg	millicurie(s)	mCi	isotopic mass number placed as	^{131}I
hour(s)	h	milliequivalent	mmol		

These and other abbreviations and symbols should follow the recommendations of: *Units, Symbols and Abbreviations*, A Guide for Biological and Medical Editors and Authors (1971) The Royal Society of Medicine, London.

Illustrations should be referred to in the text as 'Figs', and be given Arabic numbers, and should be marked on the back with the name(s) of the author(s) and the title of the paper. Where there is any possible doubt as to the orientation of an illustration, the top should be marked with an arrow. Each figure should bear a reference number corresponding to a similar number in the text. Photographs and photomicrographs should be unmounted glossy prints and should not be retouched. Colour illustrations will be accepted when found necessary by the Editor, although the author will be expected to bear the cost. Diagrams should be on separate sheets; they should be drawn with black ink on white paper on feint-ruled graph paper and should be about twice the size of the final reproduction. Lines should be of sufficient thickness to stand reduction. Each illustration should be accompanied by a legend clearly describing it; these legends should be grouped on a separate sheet of paper.

There should be as few tables as possible and these should include only essential data; they should be typewritten on separate sheets and should be given Arabic numbers.

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Page proofs will be submitted without the original typescript to the author for proof-correction (see under 'manuscripts') and should be returned to the Editor within three days. Major alterations from the text cannot be accepted.

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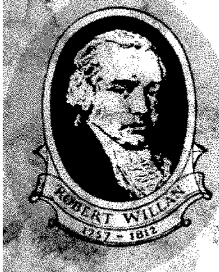

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26 Mucosal involvement in lupus erythematosus; S.M.BURGE, P.A.FRITH, F.WOJNAROWSKA, R.P.JUNIPER AND P.MILLARD
27 Bullous lesions in hydralazine induced lupus erythematosus—a review of three cases; H.J.DODD, P.M.COX AND I.SARKANY
27 Topical lithium succinate—a safe and effective treatment for seborrhoeic dermatitis in adults; D.J.GOULD, M.G.DAVIES, P.J.W.KERSEY, K.KENICER, C.GREEN, A.STRONG, E.HAMMILL, M.Y.MOSS AND Y.SMITH

28 Characteristic skin manifestations of human immunodeficiency virus (HIV) infection in asymptomatic haemophiliacs; N.R.TELFER, J.M.MATTHEWS AND F. WOJNAROWSKA

29 Immunosuppression-related skin disease in long-term transplant patients with particular reference to cyclosporin therapy; JANET MCLELLAND, A.REES, G.WILLIAMS AND A.C.CHU

29 Normal cellular immunity in Cockayne's syndrome: evidence for the role of defective immunosurveillance in the causation of skin cancer; P.G.NORRIS, A.HAMBLIN, J.COLE, C.F.ARLETT AND J.L.M.HAWK

30 The development of excess numbers of benign melanocytic naevi in children after chemotherapy for malignancy; BRONWYN R.HUGHES, W.J.CUNLIFFE AND C.C.BAILEY

31 Generalized neuropathy in neurofibromatosis: an unusual manifestation; P.K.THOMAS

32 Immunoreagent deposition in the placentae of patients with pemphigoid gestationis and linear IgA disease; S.E.KELLY, B.S.BHOGAL, F.WOJNAROWSKA AND M.M.BLACK

32 The effect of compression of skin blood flow in the lower leg; P.M.GAYLARDE, H.J.DODD AND I.SARKANY

32 Cyclic nucleotide and phosphoinositide cell regulation in psoriasis; C.B.ARCHER, S.C.CHAN AND J.M.HANIFIN

33 The clinical and pharmacological effect of lonapalene (RS-43179), a 5-lipoxygenase inhibitor, applied topically in psoriasis; A.KOBZA BLACK, R.CAMP, F.CUNNINGHAM, A.MALLET, M.HOFBAUER AND M.W.GREAVES

34 The prevalence of polycystic ovaries in patients with acne vulgaris; JULIA A.NEWTON, C.B.BUNKER, JUDY KILBORN, ANITA PATEL, H.S.JACOBS, M.W.GREAVES AND PAULINE M.DOWD

34 Diagnostic accuracy in the clinical evaluation of melanocytic lesions; REGINA K.CURLEY, R.A.MARSDEN, M.FALLOWFIELD AND M.G.COOK

35 Epithelioid cell histiocytoma—a new entity; E.WILSON JONES, R.C.CERIO AND N.P.SMITH

36 A clinicopathological and immunohistochemical study of angiolymphoid hyperplasia with eosinophilia; R.CERIO AND E.WILSON JONES

36 Nucleolar organizer region associated proteins in benign and malignant cutaneous melanocytic lesions; S.I.WHITE, M.SEYWRIGHT, H.YOUNG AND R.M.MACKIE

37 Skin and liver; P.J.SCHEUER

37 Outcome of carbon dioxide laser therapy for persistent viral warts; R.A.LOGAN AND C.B.ZACHARY

38 The treatment of recalcitrant viral warts with high dose cryosurgery under local anaesthesia; T.S.SONNEX AND R.D.R.CAMP

39 Cryotherapy for skin cancer; T.P.KINGSTON, A.HARTLEY AND P.J.AUGUST

40 Transmission of virus particles by caustic and styptic pencils and liquid nitrogen containers—a problem for dermatologists?; S.K.JONES AND J.M.DARVILLE

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41 Erythema induratum: a clinical and histological review; M.RADEMAKER, D.G.LOWE AND D.D.MUNRO

42 Calcium enhances the sensitivity of immunofluorescence for pemphigus antibodies; W.L.MATIS, G.J.ANHALT, L.A.DIAZ, E.A.RIVITTI, C.R.MARTINS AND R.S.BERGER

43 Sesquiterpene lactone mix: 6 months experience; J.S.C.ENGLISH AND I.R.WHITE

43 Genital warts and cervical intraepithelial neoplasia—a 10-year follow-up in a dermatology unit; C.J.M.STEPHENS AND F.WOJNAROWSKA

44 Investigation of the swollen limb with isotope lymphography; CHARLOTTE PROBY, P.S.MORTIMER, J.GAINE AND A.JOSEPH

45 A new method for the quantification of physical change in unstable pigmented lesions using digital image processing techniques; A.E.MURRAY, S.M.NEILL, C.C.HARLAND, C.M.E.ROWLAND-PAYNE, R.C.D.STAUGHTON AND P.W.M.COPEMAN

45 The routine use of keratin monoclonal antibodies in the differential diagnosis of cutaneous amyloid; J.R.SPAULL, R.CERIO AND E. WILSON JONES

46 Tumour necrosis factor and psoriasis; M.MURDOCH, H.NAVSARIA, F.BALKWELL, J.TROUSDALE AND I.LEIGH

46 Energy-dispersive X-ray microanalysis (EDX): a method to assess the elemental composition of hair; D.A.FENTON, I.W.MORRIS AND MARION D.KENDALL

47 Langerhans cell histiocytosis: is this a malignant process?; JULIA A.NEWTON, JANET MCLELLAND, R.S.CAMPLEJOHN AND A.C.CHU

48 Incidence of seborrhoeic dermatitis in spinal injury patients; C.L.WILSON AND M.WALSHE

48 The use of the carbon dioxide laser in dermatology; S.W.LANIGAN AND J.A.COTTERILL

49 A comparison of the efficacy and relapse rates of 311 nm phototherapy with etretinate 311 therapy (re-311) and etretinate PUVA (re-PUVA) in psoriasis therapy; C.GREEN, T.L.PATHI, B.E.JOHNSON AND J.FERGUSON

50 Elastic fibre damage with low dose penicillamine—a review of 18 patients with rheumatoid arthritis; S.BURGE, K. DALZIEL, A.MOWAT AND T.J.RYAN

50 Investigation of a low magnesium dialysate in uraemic pruritus; A.J.CARMICHAEL, M.MCHUGH AND A.M.MARTIN

51 Interferon versus bleomycin in the treatment of severe persistent viral warts—preliminary results; J.A.SALISBURY, P.MARKS AND J.R.GIBSON

52 The contribution of direct immunofluorescence (DIF) to the diagnosis of lupus erythematosus; R.E.A.WILLIAMS, R.O'KEEFE, R.M.MACKIE AND W.THOMSON

52 Which emollients should be used during phototherapy (UVB)/photochemotherapy (PUVA) for psoriasis? A study of emollient effect on minimal erythema (MED) and phototoxic (MPD) doses; J.G.LOWE AND J.FERGUSON

53 Topical retinoic acid in the treatment of solar elastotic degeneration; L.LEVER, P.KUMAR AND R.MARKS

54 Cytokine mRNA in psoriatic skin detected by *in situ* hybridization; C.B.COLVER, E.M.DICKENS, N.C.WOOD AND G.W.DUFF

54 Plasma exchange in refractory cutaneous vasculitis; N.TURNER, S. WHITTAKER, C.PUSEY AND R.RUSSELL JONES

55 Junctional epidermolysis bullosa: a clinicopathological review of 30 patients; O.M.V.SCHOFIELD, M.J.TIDMAN AND R.A.J.EADY

56 Experience of sulphamethoxypyridazine in the treatment of sub-epidermal bullous dermatoses; J.MCFADDEN, J.N.LEONARD, A.V.POWLES AND L.FRY

56 Long-term follow-up of low-dose cyclosporin treatment for psoriasis; J.MCFADDEN, A.V.POWLES, C.E.M.GRIFFITHS, B.S.BAKER, H.VALDIMARSSON AND L.FRY

57 Cutting oil dermatitis; C.E.H.GRATTAN, J.S.C.ENGLISH, I.S.FOULDS AND R.J.G.RYCROFT

57 Port wine stains: treatment with argon laser or infra-red coagulator?; S.C.MAYOU, D.A.FENTON AND D.H.MCGIBBON

58 Dermatology at Guy's Hospital: the first hundred years; J.N.W.N.BARKER, R.S.WELLS AND D.M.MACDONALD

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64 Lichen planus in a father and son; A. MILLIGAN AND R.GRAHAM-BROWN

64 Lichen planus pemphigoides; M.E.MURDOCH AND I.M.LEIGH

65 Type VII Ehlers Danlos syndrome; R.LEUNG AND R.RUSSELL-JONES

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68 Pyoderma gangrenosum and IgA paraproteinaemia—response to plasma exchange; S.J.WHITTAKER AND A.C.CHU
69 Juvenile pyoderma gangrenosum; S.C.MAYOU AND D.H.MCGIBBON
70 Scleroderma and polymyositis overlap syndrome associated with IgG lambda paraproteinaemia and cutaneous amyloid deposition; C.B.BUNKER, P.D.L.MAURICE, J.C.SHARP, A.W.P.DU VIVIER AND PAULINE M.DOWD
71 Giant cell arteritis and systemic lupus erythematosus; C.B.BUNKER AND PAULINE M.DOWD
72 Lupus erythematosus; G.DOOTSON AND I.SARKANY
72 Hypertrophic lupus erythematosus; D.C.BUCK, H.J.DODD AND I.SARKANY
74 Chronic discoid lupus erythematosus with mepacrine pigmentation bands in the nails; H.J.DODD AND I.SARKANY
75 Extensive linear epidermal naevi and polyostotic fibrous dysplasia; M.H.A.RUSTIN, C.B.BUNKER, J.J.H.GILKES, T.W.E.ROBINSON AND PAULINE M.DOWD
76 Pachydermoperiostosis with anaemia and splenomegaly; J.K.SCHOFIELD, K.GRICE AND J.KEARNEY
77 Multiple minute digitate hyperkeratoses; J.K.SCHOFIELD, K.GRICE AND N.P.SMITH
78 Scleredema of Buschke associated with multiple myeloma; J.A.SALISBURY AND I.M.LEIGH
79 Autoimmune progesterone dermatitis responding to Tamoxifen; C.J.M.STEPHENS, J.D.WILKINSON AND F.T.WOJNAROWSKA
79 Cicatricial pemphigoid, autoimmune neutropenia and thyrotoxicosis; A.L.DAVIS AND F.T. WOJNAROWSKA
80 Cicatricial pemphigoid associated with auto-immune disease; C.REID, F.WOJNAROWSKA AND P.A.FRITH
80 Chrysiasis; VANESSA VENNING, F.WOJNAROWSKA AND P.R.MILLARD
82 Argyria; G.DOOTSON, P.WILSON AND I.SARKANY
82 Xeroderma pigmentosum variant; J.BERTH-JONES AND R.A.C.GRAHAM-BROWN
83 Prolidase deficiency; A.MILLIGAN AND R.GRAHAM-BROWN
85 Giant congenital pigmented naevus; H.J.DODD AND I.SARKANY
86 Yellow nail syndrome; G.DOOTSON AND I.SARKANY
86 Conjunctival melanoma; G.DOOTSON AND I.SARKANY
87 Classical Kaposi's sarcoma and squamous cell carcinoma of the leg; G.DOOTSON AND I.SARKANY
88 Tuberous sclerosis; H.J.DODD AND I.SARKANY
89 Exfoliative cheilitis; H.J.DODD AND I.SARKANY
89 Primary facial lymphoedema with xanthomas; F.M.TATNALL AND I.SARKANY
90 Lichen aureus; C.BUCKLEY AND I.SARKANY
90 Delayed pressure urticaria, cold urticaria and chronic urticaria; FRANCES LAWLOR, A.KOBZA BLACK AND M.W.GREAVES
91 Muckle Wells syndrome in a mother and children; S.E.KELLY AND R.W.EMMERSON
92 Systemic mastocytosis with bone pain; C.B.ARCHER AND M.W.GREAVES
93 Congenital erythropoietic porphyria (Gunther's disease); C.B.ARCHER, V.S.NEILD AND J.L.M.HAWK
94 Erythropoietic protoporphyria with hepatic involvement; J.N.W.N.BARKER, R.CERIO, D.FRANCE AND D.M.MACDONALD
96 Erythropoietic protoporphyria; H.J.DODD AND I.SARKANY

96 Segmental neurofibromatosis with generalized café au lait spots; C.B.ARCHER, M.GLOVER AND D.J.ATHERTON
97 Pustular panniculitis in rheumatoid arthritis; J.NEWTON AND F.T.WOJNAROWSKA
98 Acanthosis nigricans in an hirsute woman; J.H.BARTH, D.J.EVANS AND F.WOJNAROWSKA
99 Acanthosis nigricans with insulin resistance and hyperandrogenism; D.C.BUCK, F.M.TATNALL AND I.SARKANY
100 Porokeratosis of Mibelli in an immunosuppressed patient; F.M.TATNALL AND I.SARKANY
101 Porokeratosis of Mibelli following renal transplantation; H.J.DODD AND I.SARKANY
103 Linear porokeratosis; C.BUCKLEY AND I.SARKANY
103 Tuberculosis verrucosa cutis; D.C.BUCK, H.J.DODD AND I.SARKANY
104 Dermatitis artefacta and hysterical hemiparesis; H.J.DODD, C.D.CALNAN AND I.SARKANY
105 Ossification in the interosseous membrane of the forearm after etretinate treatment for psoriasis; H.J.DODD, G.DOOTSON AND I.SARKANY
106 Xanthoma disseminatum; G.DOOTSON, J.CLARKE AND I.SARKANY
107 Junctional epidermolysis bullosa and squamous cell carcinoma; SUSAN C.PARKER, R.A.J.EADY AND M.M.BLACK
108 Onset of psoriasis associated with interferon therapy in a patient with hairy cell leukaemia; M.E.MURDOCH AND I.M.LEIGH
109 Lichen sclerosus et atrophicus of the palms and soles; K. DALZIEL, J.H.BARTH AND F.WOJNAROWSKA
109 Lichen sclerosus et atrophicus and primary biliary cirrhosis; H.J.DODD, I.SARKANY AND O.EPSTEIN
110 Localized morphoea at site of previous radiotherapy; S.M.NEILL, J.J.NICHOLL, I.W.F.HANHAM AND R.C.D.STAUGHTON
111 Mycosis fungoides and parapsoriasis lichenoides (parakeratosis variegata); S.M.NEILL, C.C.HARLAND, C.M.E.ROWLAND PAYNE, J.MC.L.SINGLETON AND R.C.D.STAUGHTON
112 Case for diagnosis: possible Cowden's disease (multiple hamartoma and neoplasia syndrome); J.ASHWORTH, P.W.M.COPEMAN, S.M.BREATHNACH AND J.J.CREAM
113 Basal cell naevus syndrome (lacking basal cell carcinomata); J.N.W.N.BARKER AND D.M.MACDONALD
114 Ulerythema ophryogenes in Noonan's syndrome; A.C.MARKEY, M.J.TIDMAN, D.E.SHARVILL AND R.S.WELLS
115 Multiple lentigines syndrome; P.D.L.MAURICE AND S.M.BREATHNACH
116 Sarcoidosis of the scalp presenting as a scarring alopecia; P.D.L.MAURICE AND S.K.GOOLAMALI
117 Necrobiosis lipoidica and sarcoidosis; H.J.DODD AND I.SARKANY

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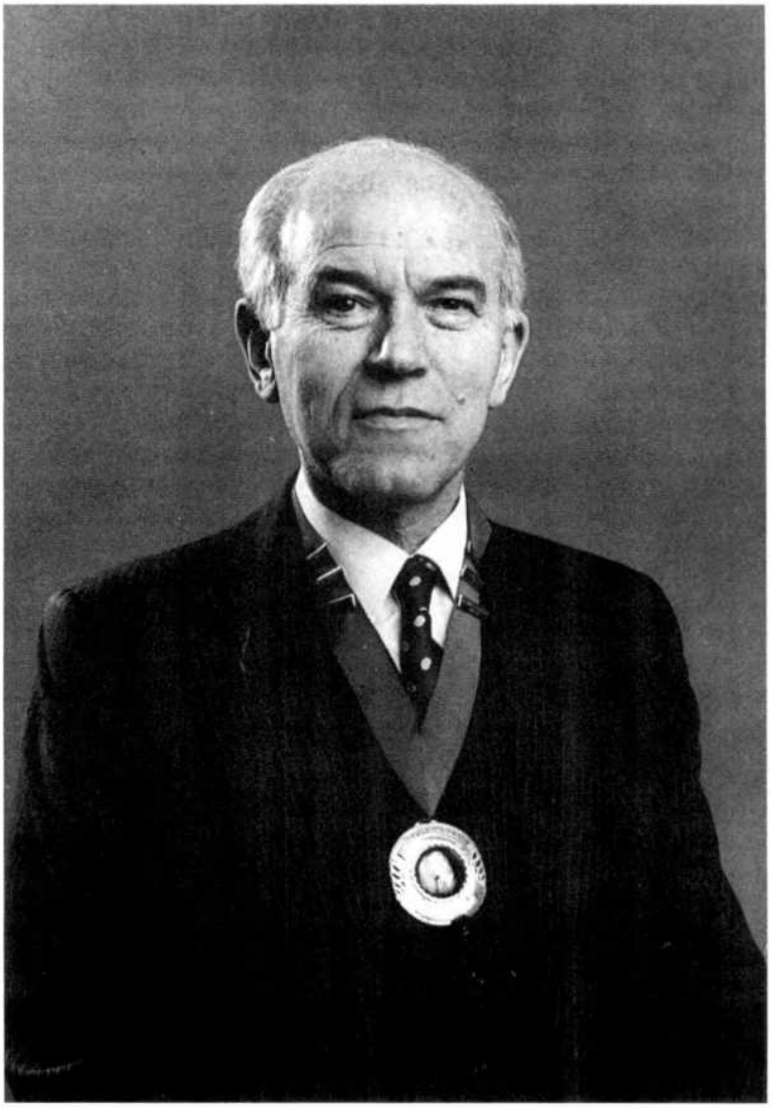
Foreword

This supplement contains details of the programme, and summaries of the papers, posters and clinical cases to be presented at the 68th Annual Meeting of the British Association of Dermatologists, to be held in London from 6 to 9 July 1988.

Once again, we are most grateful to Glaxo Laboratories for generously contributing to the cost of printing this supplement.

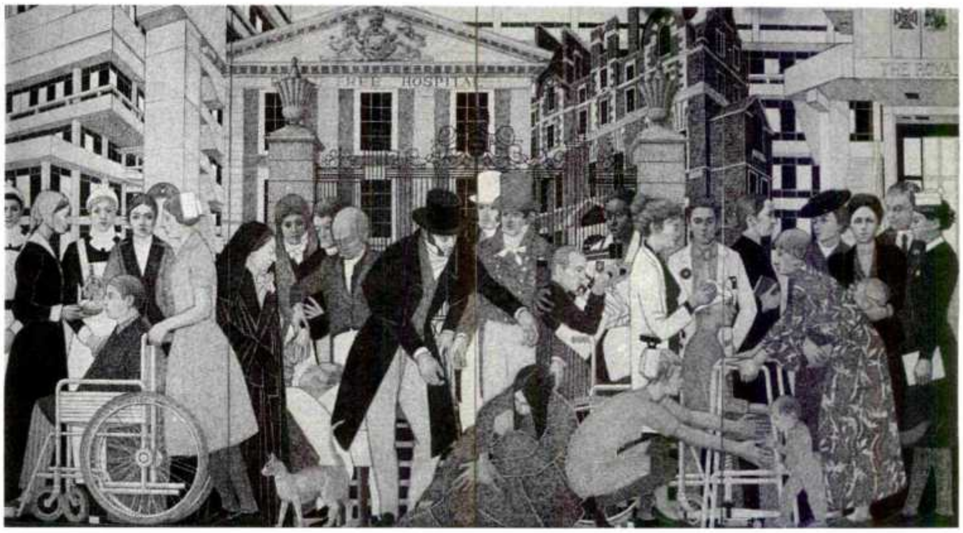
RONA M.MACKIE



DR I. SARKANY

President of the British Association of Dermatologists
1987–1988

The Royal Free Hospital, London



'The story of the Royal Free Hospital and School of Medicine' by W. Utermohlen.

This mural, depicting characters and scenes from the history of the Royal Free Hospital and the Royal Free School of Medicine, hangs in the medical school entrance.

The background depicts the new and old hospitals and the now-vacated Hunter Street medical school building—originally the London School of Medicine for Women.

Among the figures in the foreground is William Marsden, the founder of the hospital, helping a destitute girl on the steps of St. Andrew's, Holburn. Other people illustrated include: Queen Victoria, who awarded the title 'Royal'; Elizabeth Garrett Anderson, the great pioneer of women in medicine; various members of the Royal family who have been closely associated with the hospital, and students and staff of the hospital at different stages in its history.

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British Association of Dermatologists Sixty-Eighth Annual Meeting, 1988, London

PRESIDENT DR I.SARKANY

PROGRAMME OF MEETING

Wednesday, 6 July 1988

09.00	Registration commences at Kensington Town Hall
09.30	B.A.D. Annual General Meeting in the Great Hall, Kensington Town Hall (members only)
14.00–17.30	Scientific session in the Great Hall, Kensington Town Hall

Thursday, 7 July 1988

09.00–17.00	Scientific session in the Great Hall, Kensington Town Hall
18.30–20.30	President's Reception at the Natural History Museum

Friday, 8 July 1988

09.00–13.00	Scientific session in the Great Hall, Kensington Town Hall
Afternoon	Golf competition, sailing competition, visits to Kenwood House, The Wallace Collection, or Kensington Palace
19.30 for 20.00	Annual Dinner, Cafe Royal, Regent Street, London

Saturday, 9 July 1988

09.30–11.00	Viewing of patients at the Royal Free Hospital, London
10.30 onwards	Coffee
11.00–13.00	Discussion of patients
13.00	Lunch

Wednesday, 6 July 1988

09.00	Registration commences at Kensington Town Hall
09.00	Pharmaceutical Exhibition opens
09.30	Annual General Meeting (members only)
12.30	**Lunch**
12.30	**Viewing of Posters**

14.00	B.Ramsay, M.G.C.Dahl, A.J.Malcolm, H.P.Soyer and E.Wilson Jones	Acral pseudolymphomatous angiokeratoma of children (APACHE)

14.15	J.N.W.N.Barker, N.Protonatorios, A.Tsatopoulou and D.M.MacDonald	Palmoplantar keratoderma, curly hair and endomyocardial fibrodysplasia: a new syndrome
14.30	J.I.Harper, T.R.White, R.C.D.Staughton and J.R.Hobbs	Thymostimulin (TP-1) therapy for atopic eczema
14.45	G.B.Colver, J.A.Symons and G.W.Duff	Raised serum levels of soluble interleukin-2 receptor (sIL2R) in atopic eczema
15.00	S.A.Webber, R.A.C.Graham-Brown, P.E. Hutchinson and D.A.Burns	Dietary manipulation in childhood atopic dermatitis
15.15	J.Ferguson	Idiopathic solar urticaria: natural history and response to non-sedative antihistamine therapy. A study of 26 cases
15.30	**Tea**	
16.00	B.Monk and H.C.Williams	Topical diphencyprone therapy in alopecia totalis
16.15	J.H.Barth, C.A.Cherry, F.Wojnarowska and R.P.R.Dawber	Spironolactone therapy for hirsute women
16.30	R.A.Sheehan-Dare, M.J.Goodfield and N.R.Rowell	Topical PUVA and superficial X-ray therapy in chronic hand eczema
16.45	D.Shuttleworth and R.Marks	The response of precancerous lesions of the epidermis to intra-lesional α_2 interferon
17.00	R.Marks, D.Shuttleworth, S.Hill and D.M.Conelly	Relief of induced itch with a novel local anaesthetic mixture
17.15	I.M.Leigh, M.Macey and A.Newland	Treatment of recurrent erythema multiforme
17.30	**Viewing of Posters**	
18.00	Closure of Pharmaceutical Exhibition	

Thursday, 7 July 1988

08.00	Registration Desk opens	
08.15	Pharmaceutical Exhibition opens	
08.15	**Viewing of Posters**	
09.00	D.Nicholls and D.Greig	Oedemerid blister beetle dermatosis
09.15	M.Rademaker and A.Forsyth	Carba-mix: a useful indicator of rubber sensitivity
09.30	J.S.C.English, A.J.Swerdlow, R.M.MacKie, C.J.O'Doherty, J.A.A.Hunter, J.Clark and D.J.Hole	Fluorescent light and UV lamp exposure and the risk of melanoma
09.45	V.R.D'oherty, D.M.Tillman and R.M.MacKie	Congenital or early onset naevi. A risk factor for melanoma?
10.00	R.A.C.Graham-Brown, J.E.Osborne, S.M.London, A.Fletcher, P.Kirk, J.White and T.Tomlinson	The effects of a public education campaign for early diagnosis of malignant melanoma on workload and outcome—the Leicester experience
10.15	G.M.Fairris	The effect of the 1987 melanoma campaign on the workload of general practitioners and dermatologists
10.30	**Guest Lecture** S.I.Katz	The epidermal basement membrane zone—from high power to low power
11.15	**Coffee**	
11.45	M.J.D.Goodfield and N.R.Rowell	The effect of cigarette smoking on peripheral blood flow in Raynaud's phenomenon
12.00	M.Rademaker, R.H.Meyrick Thomas, J.D.Kirby and I.B.Kovacs	Effect of nifedipine on platelets and thrombolysis in systemic sclerosis
12.15	S.M.Burge, P.A.Frith, F.Wojnarowska, R.P.Juniper and P.Millard	Mucosal involvement in lupus erythematosus

12.30	H.J.Dodd, P.M.Cox and I.Sarkany	Bullous lesions in hydralazine induced lupus erythematosus—a review of three cases
12.45	**Lunch**	
14.00	D.J.Gould, M.G.Davies, P.J.W.Kersey, K.Kenicer, C.Green, A.Strong, E.Hammill, M.Y.Moss and Y.Smith	Topical lithium succinate, a safe and effective treatment for seborrhoeic dermatitis in adults
14.15	N.R.Telfer, J.M.Matthews and F.Wojnarowska	Characteristic skin manifestations of human immunodeficiency virus (HIV) infection in asymptomatic haemophiliacs
14.30	J.McLelland, A.Rees, G.Williams and A.C.Chu	Immunosuppression-related skin disease in long-term transplant patients with particular reference to cyclosporin therapy
14.45	P.G.Norris, A.Hamblin, J.Cole, C.F.Arlett and J.L.M.Hawk	Normal cellular immunity in Cockayne's syndrome: evidence for the role of defective immunosurveillance in the causation of skin cancer
15.00	B.R.Hughes, W.J.Cunliffe and C.C.Bailey	The development of excess numbers of benign melanocytic naevi in children after chemotherapy for malignancy
15.15	P.K.Thomas	Generalized neuropathy in neurofibromatosis: an unusual manifestation
15.30	**Tea**	
16.00	S.E.Kelly, B.S.Bhogal, F.Wojnarowska and M.M.Black	Immunoreagent deposition in the placentae of patients with pemphigoid gestationis and linear IgA disease
16.45	P.M.Gaylarde, H.J.Dodd and I.Sarkany	The effect of compression of skin blood flow in the lower leg
16.30	C.B.Archer, S.C.Chan and J.M.Hanifin	Cyclic nucleotide and phosphoinositide cell regulation in psoriasis

16.45	A.Kobza Black, R.Camp, F.Cunningham, A.Mallet, M.Hofbauer and M.W.Greaves	The clinical and pharmacological effect of lonapalene (RS 43179), a 5-lipoxygenase inhibitor, applied topically in psoriasis
17.00	**Viewing of Posters**	
18.00	Closure of Pharmaceutical Exhibition	

Friday, 8 July 1988

08.00	Registration Desk opens	
08.15	Pharmaceutical Exhibition opens	
08.15	**Viewing of Posters**	
09.00	J.A.Newton, C.B.Bunker, J.Kilborn, A.Patel, H.S.Jacobs, M.W.Greaves and P.M.Dowd	The prevalence of polycystic ovaries in patients with acne vulgaris
09.15	R.K.Curley, R.A.Marsden, M.Fallowfield and M.G.Cook	Diagnostic accuracy in the clinical evaluation of melanocytic lesions
09.30	E.Wilson Jones, R.C.Cerio and N.P.Smith	Epithelioid cell histiocytoma—a new entity
09.45	R.Cerio and E.Wilson Jones	A clinicopathological and immunohistochemical study of angiolymphoid hyperplasia with eosinophilia
10.00	S.I.White, M.Seywright, H.Young and R.M.MacKie	Nucleolar organizer region associated proteins in benign and malignant cutaneous melanocytic lesions
10.15	P.J.Scheuer	Skin and liver
10.30	**Coffee**	
11.00	R.A.Logan and C.B.Zachary	Outcome of carbon dioxide laser therapy for persistent viral warts
11.15	T.S.Sonnex and R.D.R.Camp	The treatment of recalcitrant viral warts with high dose cryosurgery under local anaesthesia
11.30	T.P.Kingston, A. Harley and P.J.August	Cryotherapy for skin cancer
11.45	S.K.Jones and J.M.Darville	Transmission of virus particles by caustic and styptic pencils and liquid nitrogen containers—a problem for dermatologists?

12.00	Poster Discussion
13.00	**Lunch**
14.30	Closure of Pharmaceutical Exhibition

Saturday, 9 July 1988

09.30	**Clinical Meeting at the Royal Free Hospital, London**

Poster presentations

C.Reid, F.Wojnarowska, G.Pothupitiya, J.Abooaker, B.Bhogal and M.M.Black	Chronic bullous disease of childhood around the world
M.Rademaker, D.G.Lowe and D.D.Munro	Erythema induratum: a clinical and histological review
W.L.Matis, G.J.Anhalt, L.A.Diaz, E.A.Rivitti, C.R.Martins and R.S.Berger	Calcium enhances the sensitivity of immunofluorescence for pemphigus antibodies
J.S.C.English and I.R.White	Sesquiterpene lactone mix: 6 months' experience
C.J.M.Stephens and F.Wojnarowska	Genital warts and cervical intraepithelial neoplasia—10-year follow-up in a dermatology unit
C.Proby, P.S.Mortimer, J.Gaine and A.Joseph	Investigation of the swollen limb with isotope lymphography
A.E.Murray, S.M.Neill, C.C.Harland, C.M.E.Rowland-Payne, R.C.D.Staughton and P.W.M.Copeman	A new method for the quantification of physical change in unstable pigmented lesions using digital processing techniques
J.R.Spaull, R.Cerio and E.Wilson Jones	The routine use of keratin monoclonal antibodies in the differential diagnosis of cutaneous amyloid
M.Murdoch, H.Navsaria, F. Balkwell, J.Trousdale and I.Leigh	Tumour necrosis factor and psoriasis
D.A.Fenton, I.W.Morris and M.D.Kendall	Energy-dispersive X-ray microanalysis (EDX): a method to assess the elemental composition of hair
J.A.Newton, J.McLelland, R.S.Camplejohn and A.C.Chu	Langerhans cell histiocytosis: is this a malignant process?
C.L.Wilson and M.Walshe	Incidence of seborrhoeic dermatitis in spinal injury patients
S.W.Lanigan and J.A.Cotterill	The use of the carbon dioxide laser in dermatology
C.Green, T.L.Pathi, B.E.Johnson and J.Ferguson	A comparison of the efficacy and relapse rates of 311 nm phototherapy with etretinate 311 therapy (re-311) and etretinate PUVA (re-PUVA) in psoriasis therapy
S.Burge, K.Dalziel, A.Mowat and T.J.Ryan	Elastic fibre damage with low dose penicillamine—a review of 18 patients with rheumatoid arthritis

A.J.Carmichael, M.McHugh and A.M.Martin	Investigation of a low magnesium dialysate in uraemic pruritus
J.A.Salisbury, P.Marks and J.R.Gibson	Interferon versus bleomycin in the treatment of severe persistent viral warts
R.E.A.Williams, R.O'Keefe, R.M.MacKie and W.Thomson	The contribution of direct immunofluorescence (DIF) to the diagnosis of lupus erythematosus
J.G.Lowe and J.Ferguson	Which emollients should be used during phototherapy (UVB)/photochemotherapy (PUVA) for psoriasis? A study of emollient effect on minimal erythema (MED) and phototoxic (MPD) doses
L. Lever, P.Kumar and R.Marks	Topical retinoic acid in the treatment of solar elastotic degeneration
G.B.Colver, E.M.Dickens, N.C.Wood and G.W.Duff	Cytokine mRNA in psoriatic skin detected by *in situ* hybridization
N.Turner, S.Whittaker, C.Pusey and R.Russell Jones	Plasma exchange in refractory cutaneous vasculitis
O.M.V.Schofield, M.J.Tidman and R.A.J.Eady	Junctional epidermolysis bullosa: a clinicopathological review of 30 patients
J.McFadden, J.N.Leonard, A.V.Powles and L.Fry	Experience of sulphamethoxypyridazine in the treatment of sub-epidermal bullous dermatoses
J.McFadden, A.V.Powles, C.E.M.Griffiths, B.S.Baker, H.Valdimarsson and L.Fry	Long-term follow-up of cyclosporin treatment for psoriasis
C.E.H.Grattan, J.S.C.English, I.S.Foulds and R.J.G.Rycroft	Cutting oil dermatitis
S.C.Mayou, D.A.Fenton and D.H.McGibbon	Port-wine stains: treatment with argon laser or infra-red coagulator?

Invited posters

J.N.W.N.Barker, R.S.Wells and D.M.MacDonald	Dermatology at Guy's Hospital: the first hundred years
A.Kobza Black, M.W.Greaves and A.I.Mallet	Cutaneous reactions database—Committee on Safety of Medicines Joint Collaborative Project

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SUMMARIES OF PAPERS

Acral pseudolymphomatous angiokeratoma of children (APACHE)

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Five children are described (four female, one male) who developed a unilateral eruption of multiple angiomatous papules on the extremities (in four patients on the feet and in one patient on the hand), between the ages of 2 and 13 years. The lesions were red-violaceous, discrete, irregularly shaped papules 1–4 mm in size with a hyperkeratotic collar occurring over acral sites. The provisional diagnosis in three of the patients was angiokeratoma of Mibelli, but the lesions were more numerous and chilblains were not a feature.

Histopathology showed a striking focal upper dermal lymphohistiocytic infiltrate with plasma cells, simulating pseudolymphoma, together with prominent thickened capillaries. Histochemistry in one patient showed a non-specific mixed T and B cell infiltrate in the upper dermis. The lesions have persisted, with some decrease in size, during follow-up periods of up to 14 years.

The term acral pseudolymphomatous angiokeratoma of children (APACHE) is proposed for this hitherto undescribed condition. It may represent a vascular naevus.

Palmoplantar keratoderma, curly hair and endomyocardial fibrodysplasia: A new syndrome

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Seventeen individuals (nine male, eight female; age range 9–63 years) from seven families all originating from the Greek Island of Naxos, who suffer from an inherited keratoderma associated with a cardiac anomaly, have been identified. In all pedigrees, members of a single generation only are affected with a ratio of affected to unaffected individuals of 1 : 4. The cardinal features are: palmoplantar keratoderma with an erythematous border, present by the age of 1 year; abnormal tight curly hair, present from birth; a cardiac defect. Investigations of the latter revealed a hypokinetic dilated right ventricle with an abnormal echodense band originating at the apex, a finding unique to this syndrome.

Histopathological examination of involved palmar skin in six patients revealed gross hyperkeratosis, an expanded granular layer, psoriasiform papillomatosis, premature keratinization, 'pseudo spongiosis' and mild apparent dysplasia of the basal layer. Immunohistochemical examination using monoclonal antibodies against a wide range of keratins was normal apart

from a delay of suprabasal keratin expression. Scanning electron microscopy of the hair was normal.

Two patients, because of recalcitrant ventricular tachycardia, underwent excision of the abnormal band and adjacent diseased right ventricular wall. Histological examination showed replacement of much of the ventricular wall by adipose tissue, and the band consisted largely of adipose and fibrous tissue, together with sparse hypertrophic myocardial fibres containing vacuolated sarcoplasm.

This constellation of features appears to represent a new syndrome, and the pattern of inheritance suggests an autosomal recessive trait. No other keratodermas are associated with such a specific cardiac anomaly. Because of the risk of sudden death, it is important to recognize this syndrome so that appropriate cardiological management can be established.

Thymostimulin (TP-1) therapy for atopic eczema

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Following encouraging results from a pilot study,[1] we have now completed a double-blind placebo-controlled study in 29 adults with lifelong refractory atopic eczema. Baseline data showed a significant T8 lymphopenia and a marked increase in eosinophil numbers and IgE titre; the latter two variables showed a positive correlation with clinical severity, but T8 did not. Treatment was administered by twice-weekly intramuscular injections for a period of 10 weeks (dose 1·5 mg/kg) with subsequent follow-up at 3-monthly intervals up to 1 year. At 3 months there was a median clinical improvement of 20% in those patients receiving TP-1 compared with placebo, which was statistically significant. However, this improvement was not maintained after the drug had been stopped. There was no correlation between clinical improvement and IgE, eosinophils or T8 numbers. Our results have demonstrated a beneficial effect of TP-1, but the mechanism of action is unclear.

REFERENCE

1 Staughton RCD, Byrom NA, Harper JI *et al.* Thymostimulin therapy of adult atopic eczema: a phase 1 clinical trial. In: *Thymic Factor Therapy* (NAByrom, JRHobbs, eds) New York: Raven Press, 1984; 415–23.

Raised serum levels of soluble interleukin-2 receptor (sIL2R) in atopic eczema

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The dermal infiltrate in atopic eczema includes T lymphocytes, suggesting that immune mechanisms may contribute to the pathogenesis of the disease. Activated T cells synthesize the T cell growth factor interleukin-2 (IL2) and its cell-surface receptor (IL2R). T cell clonal expansion occurs when cells bearing surface IL2R are stimulated with IL2. Soluble IL2R protein (sIL2R) is known to be released from the surface of activated T cells in proportion to its rate of synthesis.

We tested whether levels of sIL2R were raised in the sera of patients admitted to hospital for treatment of active atopic eczema. Soluble IL2R levels were measured with an ELISA using two non-competing antibodies. Preliminary results from nine patients (average age 22·4 years) with atopic eczema showed a significantly raised level compared with the serum levels in healthy age- and sex-matched controls (270 ± 30 U/ml) ($P < 0{\cdot}001$). Longitudinal studies to correlate serum sIL2R levels with disease activity are in progress.

This appears to be the first report of raised serum sIL2R levels in skin disease. As well as providing a potentially useful marker of disease activity, these results demonstrate that functional immune-cell activation occurs during atopic eczema.

Dietary manipulation in childhood atopic dermatitis

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Dietary therapy for atopic dermatitis (AD) is a controversial, but currently fashionable practice. However, there are reports that exclusion diets in children may be dangerous.[1,2] We have, therefore, studied the dietary habits of 73 children with AD seen consecutively in our out-patient clinics to ascertain the nature and frequency of dietary manipulation and to assess any potential hazards.

Although most of the children had only mild flexural AD, 71% had had significant alterations made to their diet before attendance at hospital. Almost all diets were unsupervised and only six children had seen a dietitian. Over half had been started on a diet before any professional advice had been sought. The most frequent sources of information were books, magazines, TV or radio in 37 (51%), friends 23 (32%), family doctors 20 (27%) and district nurses or health visitors 13 (18%).

The major dietary alterations included the exclusion of eggs, dairy products and food additives, and the use of soya and goats' milk. Harmful practices included the frequent use of unboiled, unpasteurized goats' milk, the use of unmodified goats' milk in a 4-month-old infant, and the use of severely restricted diets which in the long-term could have resulted in protein, calorie, vitamin or mineral deficiencies. However, there was no evidence of growth retardation or failure to thrive in our patients. Although half the children had been on a diet for more than 3 months, less than 10% of patients or parents claimed that it had been beneficial.

Dermatologists should be aware that uncontrolled, unsupervised and often prolonged dietary alterations are common in children with AD. This is a potentially serious health problem.

REFERENCES

1 Tripp JH, Francis DEM, Knight JA, Harries JT. Infant feeding practices: a cause for concern. *Br Med J* 1979; **2:** 707.

2 Tarnow-Mordi WO, Moss C, Ross K. Failure to thrive owing to inappropriate diet free of gluten and cow's milk. *Br Med J* 1984; **289:** 1113.

Idiopathic solar urticaria: natural history and response to non-sedative antihistamine therapy. A study of 26 cases

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Opinions vary as to the therapeutic value of systemic antihistamines (H_1) in the management of solar urticaria.[1,2] We have studied 26 patients with this condition, confirmed and defined by monochromator phototesting, in whom studies for porphyria were negative and a history of polymorphic light eruption or photoactive medication absent. The results showed a diverse age of onset, mean 31 years (range 1–69 years), mean duration 15·4 years (range 1–56 years). Of the 26, only two spontaneously improved in 2 and 3 years, respectively. Twenty of the 26 had used antihistamines; of these, five had complete suppression, 14 partial, and one no benefit. Strikingly, the majority of patients chose not to use this type of therapy on a regular basis because of daytime sedative effects.

Seven responders agreed to undergo phototest studies to assess the effect of terfenadine on the minimal urticarial dose at the wavelength of maximal activity (1) without therapy for at least 1 week; (2) after 3 days of terfendine 60 mg twice daily and (3) after 3 days of 120 mg twice daily. The results, as measured by an increase in minimal urticarial dose, demonstrated a highly significant benefit with 60 mg b.d. with a further, but less marked, effect with 120 mg b.d. None of these seven experienced sedation during therapy.

Solar urticaria is a persistent condition in which antihistamines are effective to varying degrees in the majority of patients. Terfenadine, a non-sedative antihistamine, appears well tolerated and is of particular value during desensitization programmes.

REFERENCES

1 Swanbeck G. Treatment of the photodermatoses. *Sem Dermatol* 1982; 1: 3.

2 Rantamen T, Suhomen R. Solar urticaria, increased mast cells and good therapeutic response to an antihistamine. *Acta Dermatovenerol* 1980; 60: 363.

Tropical diphencyprone therapy in alopecia totalis

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The therapeutic use of the induction and maintenance of allergic contact sensitization to diphencyprone (DCP) has been studied in 13 patients with alopecia totalis or universalis (AT) and two with very extensive alopecia areata (AA). The ages of the patients ranged from 10 to 46 years, and the duration of disease from 1 to 42 years (mean 10·5 years). A sensitizing dose of 2% DCP in acetone was applied to the left side of the scalp, the right side acting as an untreated control. Two weeks later a challenge dose of 0·1% DCP was applied to the left side of the scalp. Sensitization occurred in 13 of the 15 patients, and in these patients weekly applications of DCP 0·01–0·1% were applied to the left side of the scalp in order to maintain an intermittent low grade eczema. Six of the patients developed extensive regrowth of normally pigmented terminal hair, regrowth beginning after 6 to 20 weeks of treatment; the contralateral side was subsequently treated, with a positive response. Follow-up of patients for 1 year following regrowth showed that hair growth was maintained with intermittent applications of treatment. The duration of disease, associated atopy, autoimmunity, and family history appeared to have no influence on prognosis, but the development of a mild local lymphadenopathy following each application appeared to be a favourable prognostic sign. We believe that diphencyprone sensitization is a suitable therapeutic modality for selected, highly motivated patients with alopecia totalis.

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We wish to thank Drs Anthony du Vivier and Andrew Pembroke for permission to study patients under their care.

Spironolactone therapy for hirsute women

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Anti-androgens are now standard treatment for hirsuties. The most widely used regimen is combination therapy with oestrogen and cyproterone acetate. However, there are many patients who either cannot tolerate this therapy or in whom it is contra-indicated. There are subjective reports that spironolactone, which is also a potent anti-androgen, is an effective therapy for hirsuties. We have, therefore, made an objective study of its efficacy using a clinical scoring system[1] and direct measures of daily growth rates and shaft diameter of facial, forearm, abdominal and thigh hair.

We have treated 18 premenopausal hirsute women with spironolactone for a period of 12 months. This duration was chosen to ensure that the maximum improvement could be measured and to exclude any seasonal variation in hair growth.

After 3 months therapy, there was an overall mean improvement of 10% which increased to 23% after 12 months. Measures of hair diameter were significantly reduced on the abdomen and thigh ($P < 0.005$) and hair growth rates were significantly reduced on the face, abdomen and thigh ($P < 0.001$). After 12 months, there was a reduction at all sites in hair diameter ($P < 0.006$) and in growth rates ($P < 0.003$).

Side-effects were predominantly related to menstruation. Six patients developed irregular cycles, but five patients noted a regulation of menstruation. Breast tenderness was mild and only reported on direct inquiry in two patients. There was a mean weight loss of 1·4 kg and 3·4 kg after 3 and 12 months, respectively. One patient complained of gastrointestinal discomfort.

This is the most comprehensive study of the effects of spironolactone on hair growth in hirsuties. It also demonstrates that spironolactone is both an effective and well tolerated form of therapy.

REFERENCE

1 Ferriman D, Gallwey JD. Clinical measurement of body hair growth in women. *J Clin Endocrinol* 1961; **21:** 1440–7.

Topical PUVA and superficial X-ray therapy in chronic hand eczema

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Superficial X-ray therapy is of proven value in the treatment of chronic hand eczema.[1] Recent studies have suggested that systemic PUVA may also be useful in this condition.[2] Topical PUVA has not been assessed in this context. The aim of this study was to compare topical PUVA with superficial X-rays in the treatment of bilateral chronic hand eczema.

A double-blind study was performed. Patients were randomly allocated to receive superficial X-rays to one hand (three doses of 0·9 Gy using 50 kV at 21-day intervals) and topical PUVA to the other. PUVA treatment consisted of 1% psoralen solution applied 5 min prior to UVA exposure at 2 J/cm^2 increasing to 15 J/cm^2; treatments were given three times weekly for 6 weeks. Patients received sham X-ray treatment to the PUVA treated hand and sham PUVA to the X-ray treated hand to ensure that the trial was double-blind. Patients were asked to indicate severity of symptoms on a 10 cm visual analogue scale. The severity of the eczema was graded by two observers on a scale of 0–4.[1]

Fifteen out of 18 patients completed the trial. After 6 weeks treatment, the results of X-ray therapy were significantly better than those of PUVA as measured by severity grade ($P = 0·03$), but symptom scores were not significantly different. After 9 weeks there was no significant difference between the treatment modalities in severity grading or symptoms. Seven patients were followed up at 18 weeks and no significant differences were found between the two treatment modalities for severity grading or symptoms.

These results indicate that superficial X-ray therapy and topical PUVA have similar efficacy in the treatment of chronic eczema in the short to medium term. However, superficial radiotherapy may result in more rapid improvement and is less time consuming.

REFERENCES

1 Fairris GM, Mack DP, Rowell NR. Superfical X-ray therapy in the treatment of constitutional eczema of the hands. *Br J Dermatol* 1984; 111: 445–9.
2 Rosen, K, Mobacken H, Swanbeck G. Chronic eczematous dermatitis of the hands: a comparison of PUVA and UVB treatment. *Acta Dermato venereol* 1987; 67: 48–54.

The response of precancerous lesions of the epidermis to intra-lesional α-2 interferon

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Interferons are a goup of cytokines with established antineoplastic effects. Previous reports suggest that α-2 interferon may be useful in the treatment of non-melanoma skin cancer. In this study 10 patients (eight female, two male) with histologically confirmed solar keratoses or Bowen's disease were treated with intra-lesional injections of α-2 interferon twice-weekly for a period of 4 weeks. Punch biopsies (4 mm) were taken from representative areas of the lesions at

0, 4 and 8 weeks. The clinical response was monitored by measurement and by serial photography.

Clinically, the lesions became mildly inflamed by the 4th week of treatment, with complete resolution in 9 out of 10 cases by 8 weeks. The histological changes at 4 weeks consisted of a marked upper dermal inflammatory infiltrate composed predominantly of lymphocytes. In some areas the infiltrate was lichenoid and colloid bodies were present. Within the epidermis there were clusters of eosinophilic homogeneous bodies which were believed to be apoptotic cells. At 8 weeks, there was less inflammation and the dysplastic epidermal change was absent. Occasional apoptotic cells were still visible within the epidermis.

Treatment was well tolerated and only one patient developed the characteristic mild 'flu-like' symptoms during therapy. All patients have been followed up over a minimum period of 6 months and no recurrences have been observed in the areas treated. Recombinant α-2 interferon appeared to be effective in reversing epidermal changes of premalignancies, probably by a direct cytotoxic effect, although the precise mechanism by which this occurs is unclear.

Relief of induced itch with a novel local anaesthetic mixture

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Localized itch is amongst those symptoms most resistant to treatment. Persistent scratching compounds the original problem and a vicious itch-scratch cycle develops. Anaesthetic agents are effective on mucosal surfaces, but some of the '-caine' type agents used have caused sensitization. Lignocaine and prilocaine sensitize extremely rarely, and in this study the effectiveness of a eutectic mixture of these two local anaesthetics (EMLA cream) in relieving artificially induced pruritus was examined in 20 volunteer subjects. The study was conducted in two parts. In part one the effect of EMLA on the threshold concentration of histamine necessary to induce pruritus was assessed in a double-blind, placebo-controlled manner. In part two, the effect of EMLA on the perception of pruritus induced by the artificial pruritogens cowhage and papain was assessed in a single-blind manner.

Some subjects were less sensitive to histamine after placebo cream treatment, but all subjects showed marked reduction in sensitivity to histamine after EMLA treatment (Table 1). Fourteen

TABLE 1. Numbers of patients experiencing pruritus with various concentrations of histamine

	Histamine concentration ($\mu g/ml \times 10^{-4}$)									
	39	78	156	312	655	1250	2500	3300	5000	10000
Untreated site	0	0	4	9	3	2	1	1	0	0
EMLA-treated site	0	0	0	1	4	7	4	2	0	2
Placebo-treated site	0	0	0	4	7	5	2	0	1	1

of the 20 subjects required a histamine solution 2 to 3 dilutions stronger than on the untreated site to provoke a reaction. The difference between EMLA and placebo treatment was statistically significant ($P < 0{\cdot}01$).

The preparation was found to be effective in alleviating pruritus induced by cowhage and papain, and this effect was also statistically significant ($P < 0{\cdot}01$).

EMLA cream may, therefore, be useful in some clinical circumstances where persistent itch is a distressing symptom.

Treatment of recurrent erythema multiforme

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Recurrent erythema multiforme (EM) may be rare, but can be troublesome when attacks are frequent or extensive. Classical therapy includes the use of systemic steroids or immunosuppressive agents, neither option being ideal for the predominantly young patients (mean age 26). Following encouraging results with intramuscular gamma-globulin, 10 patients having very frequent attacks of EM (6–15/year) were treated with five infusions of high dose gamma-globulin over a 1 month period and their progress monitored clinically and by counts of peripheral blood lymphocyte subsets (helper, suppressor and NK cells). No consistent abnormality of T cell subsets could be found before or during therapy, but all samples normalized rapidly. Four patients were subsequently free from EM attacks for 3–6 months, two for 3 months and the remainder showed no change. Thirty-five patients with recurrent EM, 26 overtly herpes simplex virus (HSV) associated, were treated with prophylactic oral acyclovir 800 mg b.d. for 6 months and 32 had no significant HSV or EM lesions on therapy, although the problem recurred on discontinuing treatment. The effects in those patients not having overt HSV infection could be due to suppression of occult HSV or on T cell function. Thus the treatment of recurrent severe or very frequent EM warrants a trial of prophylactic oral acyclovir.

Oedemerid blister beetle dermatosis

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Blister beetle dermatosis is a distinctive vesiculobullous skin disorder and is caused by three major families of beetles (Order: Coleoptera), the eruption occurring some hours after contact with the beetle.

The best known family is the Meloidae, with reports in the literature from many parts of the world. The most notorious member is *Lytta vesicatoria*, the Spanish Fly.

The smallest family is the Oedemeridae. It is little known by comparison and given only sparse mention in the medical literature, with reports from the Pacific and the Caribbean. The vesicant chemical contained by both families of blister beetles is cantharidin. Histopathological examination of skin lesions reveals an intraepidermal vesicle.

The third group of blister beetles includes members of the genus *Paederus* (Family: Staphylinidae). The clinico-pathological picture differs owing to the vesicant agent being a different chemical, paederin. An epidemic of blister beetle dermatosis in New Zealand during January 1987 due to an endemic oedemerid beetle, *Thelyphassa lineata*, is described, and other reports of oedemerid blister beetle dermatosis in the medical and entomological literature are reviewed.

REFERENCES

1 Alexander JO. *Arthropods and Human Skin*. Springer-Verlag, Berlin: 1984; 81–2.

2 Christmas TI, Nicholls D, Holloway BA, Greig D. Blister beetle dermatosis in New Zealand. *NZ Med J* 1987; **100**: 515–17.

Carba-mix: a useful indicator of rubber sensitivity

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The value of carba-mix as a contact allergen in the European standard battery has come under question. We have analysed, retrospectively, the data from 5986 patients to determine the usefulness of carba-mix as an indicator of rubber sensitivity. Had carba-mix been excluded from the standard battery, 83 cases of contact dermatitis to rubber compounds would have been missed.

For the purposes of this study only the 96-h readings were used for analysis. Positive reactions include +, + +, and + + + reactions. Table 1 shows the total number of patients who reacted to the three rubber mixes in the European battery. Only 124 patients were carba-mix positive and negative to thiuram-mix or mercapto-mix or both. Of these, 69 were male and 55 female, mean age 46 years (range 13–87 years). One hundred and eight of these patients were tested with the carba-mix breakdown battery; 87 were positive to DPG (+ 8 irritants), 12 to ZBC, and 12 to ZDC (1 irritant). The site of the contact dermatitis was the hands (35%), face (17%), and feet (14%), with one-fifth of patients presenting with a generalized eczema. The most common identified allergens were gloves (29%), work tools (19%), footwear (13%), pillows (11%) and clothes (10%). One-half of the patients also had a positive reaction to at least one other allergen in the European standard battery.

The relevance of the carba-mix positive reaction was assessed as relevant in 58 patients,

TABLE 1.

$n = 5986$	+ve	(%)	irritants	total	(%)
thiuram-mix	383	(6·4%)	14	397	(6·6%)
mercapto-mix	162	(2·7%)	14	176	(2·9%)
carba-mix	324	(5·4%)	63	387	(6·5%)
carba-mix only	124	(2·4%)	43	167	(2·8%)

probably relevant in 25, not relevant in 10 and unknown in 31. Therefore, had carba-mix been excluded from the standard battery, 83 cases (58 + 25) of contact dermatitis to rubber compounds would have been missed (1·3% of all patients patch-tested). However, during the same period, there were 41 non-relevant carba-mix only positives (10 + 31) and 43 irritant reactions (i.e. 1·3% of all patients patch-tested). This 'false positive' rate underlines the need for good history taking and the development of a degree of expertise to allow carba-mix to be a useful test allergen.

Fluorescent light and UV lamp exposure and the risk of melanoma

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Concern has been expressed about the relationship of exposure to fluorescent light and risk of malignant melanoma.[1] Also there are reports of melanoma occurring in sunbed users and patients treated with PUVA. We, therefore, studied the risk of melanoma in relation to fluorescent light and UV lamp exposure in a case/control study of a Scottish population.

One hundred and eighty melanoma patients presenting in the years 1979–1984 and 197 control subjects were included in the study. Analysis was by stratum-matched logistic regression for age, sex and city of interview. Melanoma risk was not significantly raised for exposure to fluorescent light at home or at work. Use of UV lamps or beds, however, was associated with a significantly raised risk of melanoma (relative risk = 2·9 [95% confidence limit 1·3–6·4]) and there was a duration–response effect ($P < 0{\cdot}01$).

This is the first study to suggest that there is a link between artificial UV lamp use and melanoma.

REFERENCE

1 Beral V, Evans S, Shaw H, Milton G. Malignant melanoma and exposure to fluorescent lighting at work. *Lancet* 1982; ii: 290–3.

Congenital or early onset naevi. A risk factor for melanoma?

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We have reviewed all patients presenting with melanoma under the age of 30 in the West of Scotland between the years 1979 and 1986. In this period 1299 melanomas were registered with the West of Scotland Melanoma Group and 99 (8%) of these occurred in patients aged under 30 years at presentation. The proportion of melanoma patients under the age of 30 did not show a significant rise over this 8-year period.

Eighty-nine of the 99 patients under the age of 30 were fully assessible. They comprised 57 females and 32 males and there were no differences between the younger and older melanoma patients with regard to site of the primary lesion or average Breslow thickness. The younger patients had a higher proportion of superficial spreading melanomas (72%) in comparison with the older patients (50%).

In the course of this study it was observed that 39 (44%) of patients with melanoma under the age of 30 gave a clear clinical history of a pre-existing small congenital or early onset (before the age of 2 years) naevus on the site of the developing melanoma. In 36 of these confirmatory evidence of a pre-existing naevus was found on pathological examination. No patient in the series had a giant or garment type naevus in association with their melanoma.

A group of 92 healthy non-melanoma controls under 30 years of age were questioned and examined for historical or clinical evidence of congenital or early onset naevus. Twelve (13%) gave a positive history of such a lesion. Thus, this study shows a striking statistically significant association between small congenital or early onset naevi and development of melanoma under the age of 30 years and is further evidence of the malignant potential of small congenital naevi.

The effects of a public education campaign for early diagnosis of malignant melanoma on workload and outcome—the Leicester experience

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There is evidence that increasing public awareness may improve the proportion of thin versus thick malignant melanomas (MMs) at presentation and possibly reduce mortality.[1] As part of a project funded by the Cancer Research Campaign we have been operating an extra pigmented lesion clinic (PLC) and biopsy session each week since 1 November 1986. All general practitioners were notified when the clinic was set up but, in addition, a major local publicity campaign was conducted in Leicestershire during the week beginning 6 July 1987. We have examined our first 12 months' experience to assess the effect of this exercise on our workload and on the Breslow thickness of the MMs seen.

In 34 PLC sessions prior to publicity we saw 418 patients, an average of 12·3 per clinic. In 17 PLC sessions post-publicity we saw 926 patients, an average of 54·5 per clinic. In the two PLC sessions immediately after the campaign we saw 187 new patients. The number of MMs recorded per week rose both in Leicestershire as a whole and in the PLC during the whole of the 12-month period, but particularly after the public campaign. The total number of MMs seen in Leicestershire during the relevant 12-month period was 77, a dramatic increase on previous years (the average number for 1983, 1984 and 1985 was 34·3 per annum). Of these 39 were recorded in the 34 week pre-publicity period (15 were seen in PLC sessions: 0·44 MMs per session). The number of MMs in the 17 weeks post-publicity was 38 (21 in PLC sessions: 1·24 per session). The percentage of MMs $< 1·5$ mm rose from 64% to 81·5% in the post-publicity period, while the proportion of tumours $> 3·5$ mm fell from 23% to 2·5%.

Publicity directed at general practitioners and, more especially, at the public has resulted in a striking increase in our workload which would have been impossible to manage without extra staff. However, the weekly pick-up rate of MMs in our PLC alone rose by 181%, and there was a marked percentage shift towards 'thin' MMs throughout Leicestershire in the post-publicity period.

REFERENCE

1 Doherty VR, MacKie RM. Reasons for poor prognosis in British patients with cutaneous malignant melanoma. *Br Med J* 1986; **292:** 987.

The effect of the 1987 melanoma campaign on the workload of general practitioners and dermatologists

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A local education programme in the West of Scotland was shown to improve the prognosis of malignant melanoma. This year the Cancer Research Campaign initiated the first nationwide programme designed to educate the public to recognize early melanomas. We have measured the effect of this programme on the workload of 29 local general practitioners (GPs) and on our Pigmented Lesion Clinic (PLC). In addition, we measured the effect of education on the percentage of patients with pigmented lesions referred by GPs to our PLC.

Before the campaign all 241 local GPs were sent an illustrated booklet about the diagnosis of pigmented lesions and 150 attended a lecture on melanoma. The education programme was launched in July and was covered as a news item by the media.

Before July, 1·7 patients per day consulted these GPs about a pigmented lesion. After July this number rose significantly to 5·3 patients per day. Education of the GPs significantly reduced the percentage of those patients who were referred to the PLC from 53% to 37%. The number of patients referred to the PLC during the 5 months following the programme increased 2·8-fold compared with the previous year. Every attempt was made to limit the amount of surgery carried out. Even so, a minor surgical procedure was performed on 242 of the 750 patients seen during these months.

In the 5 months since the campaign 20 melanomas have been diagnosed in the PLC. Nine were superficial spreading melanomas with a mean thickness of 0·65 mm (95% confidence interval 0·39–0·91 mm).

The national campaign significantly increased the workload of both GPs and dermatologists in Southampton. However, it produced a rewarding number of thin, good prognosis melanomas. Education of the local GPs reduced the percentage of patients with pigmented lesions who needed to be referred to the PLC.

The effect of cigarette smoking on peripheral blood flow in Raynaud's phenomenon

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It is known that cigarette smoking reduces cutaneous blood flow,[1] but there is little information concerning the effect of smoking on finger blood flow in patients with Raynaud's phenomenon.

We have measured finger pulp blood flow (mainly A-V shunt flow) and dorsal finger flow (mainly capillary flow) in patients with Raynaud's phenomenon and in normal controls. Subjects were divided into regular (every day) smokers, occasional (not every day) smokers and non-smokers. All subjects abstained from smoking for 12 h, and measurements were made in a controlled environment at 24°C.

Baseline measurements of blood flow were made using a laser Doppler flowmeter sited at the tip of the third finger of the right hand, and in the second dorsal finger-web space. A single cigarette (middle tar) was smoked by each subject who was asked to inhale deeply, and then blood flow measurements were repeated.

In regular smokers in both groups, finger pulp blood flow fell by a mean of 2·4 (range 0·5–4·5) ($P < 0{\cdot}05$), and remained depressed for up to 2 h. The magnitude of the reduction tended to be greater in subjects with RP. In occasional smokers and non-smokers, there was no change in finger pulp blood flow. Dorsal finger web flow was unchanged in all groups.

The difference in response of blood flow at the two sites suggests that smoking-induced vasoconstriction occurs most in the pre-A-V shunt arterioles, and the response in regular smokers suggests that regular smoking sensitizes these vessels to vasoconstricting stimuli. In view of the prolonged reduction in finger blood flow as the result of smoking only one cigarette, regular smokers with Raynaud's phenomenon should be advised to stop altogether, not just to reduce smoking.

REFERENCE

1 Richardson D. Effects of tobacco smoke inhalation on capillary blood flow in human skin. *Arch Environ Health* 1987; **42**: 19–25.

Effect of nifedipine on platelets and thrombolysis in systemic sclerosis

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Although the pathogenesis of systemic sclerosis remains unclear, there is increasing evidence that a vascular defect, both morphological and functional, is important in the progression of the disease. Recent work has indicated that there is both an activation of platelets and disturbance of thrombolysis in patients with systemic sclerosis.

We have investigated this further with the aid of a newly-designed instrument, the haemostatometer, which measures the pattern and speed of haemostatic plug formation (HPF) following the puncturing of polyethylene tubing through which non anti-coagulated blood flows under standard conditions. The pattern and speed of whole blood coagulation (CT) and spontaneous thrombolysis (STT) were also measured.

Thirty-five patients with systemic sclerosis and 30 matched controls were studied. In a further 14 patients, measurements were made before and 90 min after 10 mg oral nifedipine.

Patients with systemic sclerosis had significantly shorter haemostatic plug formation times than the controls (2·2 ± 0·2 min and 3·2 ± 0·2 min, respectively, $P = 0{\cdot}0003$) indicating activation of platelets, longer coagulation times that the controls (14·2 ± 0·7 min and 11·9 ± 0·7 min, respectively, $P = 0{\cdot}025$) indicating a disturbed coagulation system, and longer spontaneous thrombolysis times than the controls (57·7 ± 2·8 min and 35·7 ± 2·3 min, respectively, $P = 0{\cdot}00001$) demonstrating alteration in fibrinolysis.

Nifedipine had a significant normalizing effect on both HPF (2·4 ± 0·2 min to 2·9 ± 0·4 min, $P = 0{\cdot}0057$) and STT (57·7 ± 2·8 min to 34·2 ± 5·3 min, $P = 0{\cdot}014$) in the 14 patients studied after oral nifedipine. It had no effect on coagulation.

These results indicate that patients with systemic sclerosis have activated platelets, impaired coagulation, and altered thrombolysis and fibrinolysis. This may be of importance in the pathophysiology of systemic sclerosis. It is of interest that nifedipine appears to correct these abnormal changes and it is possible that this is the mechanism of action in the improvement in Raynaud's phenomenon in patients with systemic sclerosis seen in clinical trials with calcium antagonists.

Mucosal involvement in lupus erythematosus

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Mucosal involvement in lupus erythematosus (LE) is poorly documented and the immunopathology of conjunctiva in LE has not previously been reported. The prevalence of clinical and immunopathological mucosal involvement has been studied in 38 patients with systemic LE (SLE) and 42 patients with discoid LE (DLE). Oral and nasal mucosa were examined and a full ocular assessment, including slit lamp examination was performed. Biopsies were taken from oral labial mucosa (47), conjuctiva (19) and non-exposed forearm skin (52) and the presence of immunoreactants detected by direct immunofluorescence.

Clinical mucosal involvement was a feature in 45% (17 of 38) SLE patients and 48% (20 of 42) DLE patients. Intermittent mouth ulcers were a feature in one-third of both SLE and DLE patients. Six patients (five DLE) had plaques on buccal mucosa (asymptomatic in five); seven patients (five DLE) had nasal involvement, episcleritis was a presenting feature in one SLE patient, and two DLE patients had lower eyelid scarring with loss of lashes, scarring of the lower conjunctival sac and symblepheron.

A linear band of immunoreactants (the lupus band) was present in 25% of skin biopsies, 9% of oral biopsies and 63% of conjunctival biopsies. In DLE, conjunctiva was frequently positive when skin and oral mucosa were negative.

Clinical mucosal involvement is a feature of DLE and to a lesser extent of SLE, but it is often unrecognized. In addition, a lupus band may be detected in clinically uninvolved conjunctiva.

Clearance of immunoreactants may be slower from conjunctiva than from other sites as has been suggested in pemphigoid.[1]

REFERENCE

1 Venning VA, Frith PA, Bron AJ *et al.* Mucosal involvement in bullous and cicatricial pemphigoid. A clinical and immunopathological study. *Br J Dematol* 1988; 118: 7–15.

Bullous lesions in hydralazine induced lupus erythematosus—a review of three cases

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Large, tense, thick-roofed blisters were a prominent feature of hydralazine induced lupus erythematosus in three patients, aged from 65 to 74 years, who were receiving between 50 and 150 mg daily for hypertension. The initial clinical diagnosis included bullous pemphigoid (one case) and erythema multiforme (one case), but histology and the development of subsequent lesions showed that the blistering was due to an underlying vasculitis in each case. Two patients had deep lingual ulcers at the time of presentation and two patients had previously undergone extensive investigations for other symptoms and signs which were also due to hydralazine induced LE. These included anaemia, pleural effusions, neutropenia, thrombocytopenia and splenomegaly. All patients had a diffuse pattern of antinuclear antibodies and elevated DNA binding.

The blistering was followed by significant areas of skin necrosis in all three patients. In two patients this was complicated by serious secondary bacterial infection and one eventually required mid-thigh amputation.

Blistering is an uncommon feature of drug induced lupus erythematosus. Its recognition may aid the early diagnosis of this serious drug reaction.

Topical lithium succinate—a safe and effective treatment for seborrhoeic dermatitis in adults

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Seventy-six adult patients with a diagnosis of seborrhoeic dermatitis, from four centres, were entered into a randomized, double-blind, placebo-controlled, crossover study to investigate the efficiency and safety of lithium succinate ointment (8% lithium succinate, 0·5% zinc sulphate and 0·1% preservative in wool alcohols), over a 10-week period.

Sixty-four patients completed the study and a statistically significant benefit produced by active treatment was observed in all parameters of seborrhoeic dermatitis, using the sign test.

Parameters measured included redness, scaling, itching, greasiness, overall improvement and global assessment.

No haematological or biochemical abnormalities were detected during the study. Serum lithium and plasma zinc levels remained unchanged as a result of treatment.

Two patients withdrew following a marked skin reaction to the placebo ointment. Patch-testing did not confirm this as an allergic contact dermatitis.

This study confirms the findings of an earlier, smaller, controlled trial and furnishes evidence of the safety of the preparation. The mode of action remains conjectural. Lithium succinate may have a general anti-inflammatory effect, but does not appear to inhibit the growth of *P. ovale*, which has been implicated in the aetiology of seborrhoeic dermatitis.

REFERENCE

1 Boyle J, Burton JL, Faergemann J. Use of topical lithium succinate for seborrhoeic dermatitis. *Br Med J* 1986; **292**: 28.

Characteristic skin manifestations of human immunodeficiency virus (HIV) infection in asymptomatic haemophiliacs

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The prevalence of cutaneous pathology in haemophiliacs infected by HIV has not previously been studed. All previous studies of this problem have dealt chiefly with homosexual males, intravenous drug abusers, and sexual contacts of these two groups. These patients differ markedly in both age distribution and lifestyles from haemophiliacs. We have studied 41 patients routinely attending the Oxford Haemophilia Centre. Each patient was examined by a single observer who was unaware of their HIV antibody status. Twenty-four patients were antibody positive (age range 16–70 years, mean 38·6 years). No patient had AIDS or AIDS-related complex (ARC). There was a significant difference between the HIV positive and HIV negative patients in the prevalence of seborrhoeic dermatitis, folliculitis, mucocutaneous candidiasis and dermatophyte infection ($P < 0{\cdot}005$; chi-squared test). Eleven patients had seborrhoeic dermatitis (10 HIV + ve), eight folliculitis (six HIV + ve), four mucocutaneous candidiasis (all HIV + ve) and three culture proven dermatophyte infection (all HIV + ve). This series of haemophiliacs has shown an increased prevalence of skin diseases in the HIV + ve group compared with HIV − ve haemophiliacs and with age-matched normal controls. These diseases detected are well recognized cutaneous manifestations of AIDS and ARC. This study demonstrates their increased prevalence in otherwise asymptomatic HIV + ve patients when compared with controls.[1]

REFERENCE

1 Muhlemann MF, Anderson MG, Paradines FJ *et al.* Early warning skin signs in AIDS and persistent generalized lymphodenopathy. *Br J Dermatol* 1986; **114**: 419–24.

Immunosuppression-related skin disease in long-term transplant patients with particular reference to cyclosporin therapy

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One hundred and twenty-one patients who had received a renal allograft between 4 months and 21 years previously (mean ± SD 71 ± 62 months) were studied. Seventy-two patients were conventionally immunosuppressed with azathioprine and prednisolone, 36 had been given the current regimen of cyclosporin, azathioprine and prednisolone and 13 had received other combinations of drugs.

Forty-five patients had viral warts, of whom 20 had more than 10 warts. The presence of viral warts was significantly associated with pale skin type, excess sun exposure and with duration of allograft. Viral warts were significantly more common in those on conventional immunosuppressive therapy, but this could be solely a reflection of the difference in duration of transplant between the two groups. Seven per cent had fungal skin infections which were significantly more common in patients with skins that tanned easily than in those who burned in the sun. Twelve patients were found to have developed dysplastic or neoplastic skin lesions since transplantation. The incidence of dysplasia increased with increasing age and was significantly associated with pale skin type, excess sun exposure and duration of allograft. Despite the shorter duration of treatment in those on the new treatment regimen, there was no difference between the two groups in the proportion of patients with dysplastic skin lesions.

Immunosuppression-related skin disease may be a significant problem in allograft recipients in this country and we suspect that patients taking cyclosporin will have similar problems to those on conventional immunosuppressive drugs alone.

Normal cellular immunity in Cockayne's syndrome: evidence for the role of defective immunosurveillance in the causation of skin cancer

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There is evidence of defective DNA repair and increased UV-induced fibroblast mutability in both Cockayne's syndrome (CS) and xeroderma pigmentosum (XP), but increased cancer risk occurs only in the latter. XP patients also have defective cell mediated immunity (CMI),[1] but this has not previously been studied in CS.

Three girls with CS (aged 2·5–14 years), diagnosed by appropriate clinical features and by impaired post-UV RNA synthesis, were investigated. Frequency of mutation of circulating lymphocytes to resistance to the lethal action of 6-thioguanine ($6TG^{Res}$ MF), a measure of somatic cell mutation rate, was assessed using a modified cloning technique.[2] In two patients CMI was studied by means of 48 h cutaneous hypersensitive responses to seven different recall antigens, measurement of circulating T cell subsets, and proliferative lymphocyte responses to tuberculin, *Candida* and phytohaemagglutinin. Mean $6TG^{Res}$ MF was approximately 7×10^{-6}, significantly above normal and similar to that seen in children in the same age group with XP.

However, in contrast to findings in XP, all measures of immune function were within normal limits. Thus, in CS, lack of increased skin cancer susceptibility despite high somatic cell mutation rate may be due to normal CMI.

REFERENCES

1 Wysenbeek AJ, Weiss H, Duczyminer-Kahana M *et al.* Immunologic alterations in xeroderma pigmentosum. *Cancer* 1986; **58:** 219–21.

2 Henderson L, Cole H, Cole J *et al.* Detection of somatic mutations in man; evaluation of the microtitre cloning assay for T-lymphocytes. *Mutagenesis* 1986; **1:** 195–200.

The development of excess numbers of benign melanocytic naevi in children after chemotherapy for malignancy

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In nine patients attending a paediatric oncology clinic who had successfully completed a 2-year course of chemotherapy for acute lymphatic leukaemia or lymphoma, the appearance of large numbers of pigmented naevi (mean 72; range 18–183) was noted at the time of completion of treatment. The numbers were greatly increased compared to the normal population.[1]

A blind study was, therefore, undertaken to compare the numbers of moles in patients on chemotherapy with those who had completed treatment. All patients were seen between November and February to minimize the problem of solar stimulation of freckles. All moles were counted by the same observer and noted to be either $<$ or $\geqslant 3$ mm diameter. Lesions known to be present since birth were excluded.

Sixty-six patients, mean age 9 years (range 4–17 years) were matched for age, sex and race. The diagnoses in these patients were acute lymphatic leukaemia (52 patients), lymphoma (nine patients) and rhabdomyosarcoma (five patients). The mean time after completion of treatment in 33 patients was 28 months.

There was a significantly higher mole count in those patients who had completed therapy, both total count ($P < 0{\cdot}001$), and moles $\geqslant 3$ mm ($P < 0{\cdot}001$); 56% of those who had completed treatment and 18% of those on treatment had moles on acral sites.

Patients who have undergone radical chemotherapy are at risk of a second malignancy. Melanoma has been shown to be strongly related to numbers of benign melanocytic naevi.[2]

We report this important finding as a group of patients who may be at risk of future melanoma and also as a group of patients where there is an opportunity for further study of the biology of the benign naevus.

REFERENCES

1 MacKie RM, English J, Aitchison TS *et al.* The number and distribution of benign pigmented moles (melanocytic naevi) in a healthy British population. *Br J Dermatol* 1985; **113:** 167–74.

2 Swerdlow AJ, English J, MacKie RM *et al.* Benign melanocytic naevi as a risk factor for malignant melanoma. *Br Med J* 1986; **292:** 1555–9.

Generalized neuropathy in neurofibromatosis: an unusual manifestation

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Neurofibromatosis consists of two genetically distinct conditions: von Recklinghausen's neurofibromatosis (VRNF) and bilateral acoustic neurofibromatosis (BANF). The occurrence of neuromas on the eighth cranial nerves or on spinal roots in BANF gives rise to neurological deficits but, except sometimes for lesions in the limb girdle plexuses or if malignant change occurs, deficits from neurofibromata on peripheral nerves are rare in both VRNF and BANF.

I have seen three cases of neurofibromatosis that presented with the onset in adult life of a slowly progressive distal sensorimotor neuropathy. Two had VRNF and the third had BANF. In the patient with BANF, a chromosome 22 deletion was demonstrated both in cells in his acoustic neuroma and in a cutaneous neurofibroma. All three cases showed generalized nodular enlargement of the peripheral nerves and neurofibromatous changes on nerve biopsy. In the patient with BANF, an unusual histological feature was the presence of large formations composed of concentrically arranged Schwann cells, similar to the appearances described in a single previously recorded case of 'neurofibromatosis neuropathy' in BANF by Ohnishi and Nada.[1]

A generalized distal sensorimotor neuropathy is a rare manifestation of neurofibromatosis.

REFERENCE

1 Ohnishi A, Nada O. Ultrastructure of the onion-bulb like lamellated structures observed in the sural nerve and in a case of von Recklinghausen's disease. *Acta Neuropathol (Berl)* 1972; **20**: 258.

Immunoreagent deposition in the placentae of patients with pemphigoid gestationis and linear IgA disease

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Skin and amnion share a common ectodermal origin with evidence of shared antigenic structures. In the bullous disorders, linear IgA disease (LAD) and pemphigoid gestationis (PG), antibodies are directed against antigenic determinants present in the cutaneous basement membrane zone (BMZ). These antibodies have been shown by indirect methods to cross-react with antigens present in the normal human placenta from the second trimester of pregnancy until term.

We have followed pregnancies in two adult females with LAD and in five with PG. At the time of delivery the placentae from the patients were obtained and examined histologically and immunologically. A direct immunofluorescence (IF) assay of *in vivo* immunoreagent deposition was employed using standard FITC labelled antibodies to IgG, IgA and complement fragments, and monoclonal antibodies to the IgG subclasses. All the babies were healthy without evidence of any blistering disease. The placentae varied in weight and were morphologically normal, with normal placental histology and no evidence of blister formation. Direct IF revealed a linear band of IgG_1 in the BMZ of all the PG placentae. Complement deposition was evident in all five pregnancies with evidence of activation of the classical complement pathway. In both LAD placentae IgA was deposited in a linear band in the amniotic BMZ, but there was no evidence of complement activation.

This study documents the first demonstration of these immunoreactants in the placenta in PG and LAD. In these conditions uninvolved placenta, like uninvolved skin, shows evidence of immunoreactant deposition.

The effect of compression of skin blood flow in the lower leg

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It has long been established that bandaging or elastic compression has a favourable influence on venous leg ulcers. Compression of the lower leg using an inflatable splint increases skin oxygen tension (PO_2) in the standing position and diminishes it in recumbent subjects. The change in PO_2 varies with pressure and in this way the optimal pressure required for treatment may be established.

In patients with venous leg ulcers, venous distension in the upright position stimulates stretch receptors in the veins, and induces arteriolar vasoconstriction via a spinal reflex. This in turn leads to hypoxia which is the immediate cause of leg ulceration. This study shows that venous compression in the lower legs inhibits the vasoconstriction reflex responsible for hypoxia. The therapeutic role of bandaging is thereby explained.

Cyclic nucleotide and phosphoinositide cell regulation in psoriasis

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Abnormalities of cyclic nucleotide and phosphoinositide (PI) metabolism in psoriatic epidermis have been implicated in the pathogenesis of psoriasis.[1,2] We have investigated the cyclic nucleotide and PI cell regulatory pathways which may underlie immune dysfunction in psoriasis, by measuring peripheral blood mononuclear leukocyte (MNL) cyclic AMP-phosphodiesterase (PDE) activity and phospholipase C (PLC) activity.

Using Ficoll-Hypaque separated MNLs from 27 normal and 17 psoriatic subjects, PDE activity was determined on cell homogenates by radioenzyme assay. For PLC assay, endogenous PIs from normal MNLs, labelled overnight with gamma ^{32}P-ATP, served as substrate using the cytosol fractions of normal and psoriatic MNLs as the PLC source. Total PI conversion and individual PI hydrolysis to inositol phosphates were measured and PDE activity was determined on these cell populations ($n = 8$).

Mean PDE activity, expressed as pmol cAMP/min/10^6 cells ($\pm$SEM), was significantly increased in psoriatic cells ($0{\cdot}114 \pm 0{\cdot}014$) compared with normal cells ($0{\cdot}037 \pm 0{\cdot}001$) ($P < 0{\cdot}001$). There was greater conversion both of total PI and individual PI fractions in samples from psoriatics, implying increased PLC activity. In addition, there was a correlation between MNL PDE activity and MNL cytosol PLC activity ($r = 0{\cdot}9$).

These studies suggest a link between cyclic nucleotide and phosphoinositide regulatory abnormalities which may lead to immune and proliferative aspects of cell dysfunction in psoriasis.

REFERENCES

1 Royer E, Chaintreul J, Meynadier J *et al.* Cyclic AMP and cyclic GMP production in normal and psoriatic epidermis. *Dermatologica* 1982; **165**: 533–45.

2 Bartel RL, Marcelo CL, Voorhees JJ. Partial characterization of phospholipase activity in normal, psoriatic uninvolved and lesional epidermis. *J Invest Dermatol* 1987; **88**: 447–51.

The clinical and pharmacological effect of lonapalene (RS-43179), a 5-lipoxygenase inhibitor, applied topically in psoriasis

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As 5-lipoxygenase products of arachidonic acid (AA) may have a pathogenetic role in psoriasis, the effects of a 5-lipoxygenase inhibitor, lonapalene, were studied in 10 patients with psoriasis.

Two per cent lonapalene ointment and its vehicle base were applied twice daily to symmetrical plaques in a double-blind manner, with other plaques receiving no treatment. Erythema, induration and desquamation were assessed visually at 0, 4, 14 and 28 days and lesions sampled at 4 and 14 days by a skin chamber technique.

Clinical grading showed significant improvement in lonapalene treated lesions compared with untreated lesions at 4, 14 and 28 days (Mann-Whitney U test). While there was a trend towards greater improvement in lonapalene compared with vehicle treated lesions, this was not statistically significant because of substantial vehicle effects. One patient withdrew because of lonapalene induced skin irritation. Chamber fluid was analysed for leukotriene B_4 (LTB_4) by high performance liquid chromatography (HPLC) and assay of selected fractions for neutrophil chemokinesis and 12-hydroxyeicosatetraenoic acid (12-HETE) and arachidonic acid (AA) by HPLC followed by gas chromatography–mass spectrometry. Only LTB_4 was significantly reduced in samples from lonapalene treated lesions compared with vehicle treated and untreated lesions; at 4 days untreated 59 ± 12 pg/ml, vehicle 73 ± 10, lonapalene 27 ± 11 ($P < 0{\cdot}01$); at 14 days, untreated 53 ± 17, vehicle 74 ± 9, lonapalene 19 ± 6 ($P < 0{\cdot}01$) (paired t-test). AA and 12 HETE levels remained unchanged.

These results suggest that topically applied lonapalene, previously shown to be clinically effective in psoriasis,[1] may act in part by selective inhibition of the 5-lipoxygenase pathway resulting in reduced LTB_4 formation, as demonstrated in this study.

REFERENCE

1 Lassus A, Forsstrom S. A dimethoxynaphthalene derivative (RS-43179 gel) compared with 0·025% fluocinolone acetonide gel in the treatment of psoriasis. *Br J Dermatol* 1985; **113**: 103.

The prevalence of polycystic ovaries in patients with acne vulgaris

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Acne vulgaris is recognized as an androgen dependent disorder. The historical concept of the polycystic ovary syndrome is of an uncommon association of infertility, obesity and hirsutism, with occasional acne. However, using modern high resolution ultrasound, it has been shown that polycystic ovaries occur in 95% of cases of hirsutism and 85% of cases of oligomenorrhoea.[1] Indeed, the polycystic ovary pattern is seen in up to 20% of the normal population. We have, therefore, determined the prevalence of ultrasonically defined polycystic ovaries in a group of acne patients.

A total of 100 consecutive female patients with acne (mean age 25 years, range 14 to 40 years), and 10 clinically normal controls who denied a history of acne (mean age 33 years, range 24 to 39 years) were recruited. The severity of the acne was graded and patients were examined for other features of the polycystic ovary syndrome. High resolution ultrasound scans of the pelvis were performed between days 6 and 10 of the menstrual cycle (mid follicular phase), and polycystic ovaries were diagnosed according to the criteria of Adams *et al.*[2] Estimation of LH, FSH, testosterone, prolactin and thyroxine were performed on the same day.

A total of 66 acne patients attended for scans. Fifty-two (79%) showed a definite polycystic pattern, and 10 had a borderline anomaly. The presence of the polycystic pattern was not restricted to patients with severe acne, clinical features of the polycystic ovary syndrome, or marked hormonal abnormality. Of the 10 controls, only three had a polycystic pattern on ultrasound scan.

This study indicates a very high prevalence of polycystic ovaries in women with acne vulgaris.

REFERENCES

1 Adams J, Polson DW, Franks S. Prevalence of polycystic ovaries in women with anovulation and idiopathic hirsutism. *Br Med J* 1986; **293**: 355–9.

2 Jacobs HS. Review. Polycystic ovaries and the polycystic ovary syndrome. *Gynecol Endocrinol* 1987; **1**: 113–31.

Diagnostic accuracy in the clinical evaluation of melanocytic lesions

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Previous studies of the prevalence of benign and dysplastic melanocytic naevi have assumed that such lesions can be diagnosed on clinical grounds. But is this so?

Prior to a survey of dysplastic naevi in the community we tested our ability to recognize different types of melanocytic lesion. One hundred and sixteen consecutive and apparently benign pigmented lesions were removed for diagnostic purposes from 80 patients attending the pigmented lesion clinic. Before removal they were assessed independently by up to three experienced observers. Specimens were sent for histology in coded bottles without clinical details. The findings were not made available until the end of the study, except when necessary for the continuing management of the patient. All specimens were re-coded and re-submitted to the same pathologist for a second evaluation.

The final histological diagnoses were melanoma (3), dysplastic naevus (33), compound naevus (19), intradermal naevus (28), lentigo (6), seborrhoeic wart (8), and miscellaneous (19).

Overall, only half the lesions were diagnosed correctly. Diagnostic accuracies for the three observers were 45%, 50%, and 52%. All three melanomas were misdiagnosed by at least one observer. Only five of the 28 intradermal and four of the 19 compound naevi were correctly diagnosed by all observers. Of the 33 dysplastic naevi, 18 were correctly diagnosed by all observers, but a further 24 banal lesions were falsely interpreted as dysplastic by one or more observers.

The findings show that it is extremely difficult to distinguish between certain pigmented lesions. Clearly epidemiological studies of dysplastic naevi must not be based on clinical recognition alone; histological confirmation is necessary.

Epithelioid cell histiocytoma—a new entity

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We report a previously undescribed variant of histiocytoma which is distinguished by its occurrence as an elevated, fleshy nodule confined to the superficial dermis and cytologically by the presence of characteristic, large, angulated epithelioid cells. We believe that this lesion may have been misdiagnosed histologically as a Spitz naevus or melanoma in the past. Nineteen specimens from 18 patients (one was a recurrent lesion) have been studied. There were 10 females and eight males with a mean age at presentation of 39 years (range 23–63). The lesions were slow-growing and painless, some reaching 1·5 cm in size before excision. The clinical differential diagnosis was most commonly that of a resolving pyogenic granuloma. All occurred on the limbs, particularly the thigh or leg, except for one on the shoulder.

Histologically the lesion occurred as a sessile or pedunculated mass, often with a collar of thickened epidermis partially enclosing the base. The overlying epidermis was attenuated and no lesions were ulcerated. The predominant cells were large epithelioid cells with a prominent eosinophilic cytoplasm. In most specimens, binucleate and trinucleate cells were seen, but large bizarre multinucleate cells were not a feature. Nuclear atypia and mitotic activity were absent. Vascularity was prominent in all but two lesions. Spindle cells were observed, with areas of transition to 'ordinary' histiocytoma in five lesions. Blood pigment deposition was encountered in a few of the lesions, but no melanin was seen. Immunohistochemical findings mirrored those found in 'ordinary' histiocytoma. In particular, approximately 50% of the constituent cells were positive for factor XIIIa. Only a minority of the cells were positive for S100 protein and macrophage markers. None were positive for cytokeratins. The vascularity was emphasized by endothelial cell markers. Recognition of this benign tumour-like lesion should help prevent a misdiagnosis of Spitz naevus.

A clinicopathological and immunohistochemical study of angiolymphoid hyperplasia with eosinophilia

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The clinicopathological data from 103 cases of angiolymphoid hyperplasia with eosinophilia (ALHE) have been analysed. The patients included 70 females and 33 males, with a mean age at presentation of 34 years. Most cases presented with either superficial multiple angiomatous papules around the ears or with deep-seated single or multiple nodules on the scalp, face or neck. Unusual sites of involvement included the popliteal fossa, penis, salivary tissue, trapezium muscle and finger. In nearly half the patients with subcutaneous lesions there was involvement of large arterial vessels.

In order to analyse the immunolabelling of the abnormal vessels and cellular infiltrate, an immunohistochemical study using 12 cell markers was performed on 21 biopsy specimens from 13 selected cases. Both superficial and deep lesions were included. Endothelial cell markers included F VIII RA, lectin UE Agg-1, EN4 and PAL E. Inflammatory cell markers included UCHL1 (T cells), 4KB5 (B cells), MAC 411 (monocyte dermal macrophages), DA 6.231 (Class II antigen), S100 protein (Langerhans cells), F XIIIa (dermal dendrocytes), chloroacetate esterase (mast cells and neutrophil precursor) and vimentin (mesenchymal cells).

The results showed labelling of the abnormal vessels in ALHE with all endothelial cell markers. There was some loss of immunoreactivity with F VIII RA and PAL E compared with UE Agg-1 and EN4 suggesting that the predominant vessels are venular capillaries. Approximately 80% of the mononuclear cells were T cells and 15% B cells. Follicular formation resembling early germinal centres was only seen in deeper lesions. Small numbers of MAC 411, S100 and chloroacetate esterase positive cells were also found. The number of dermal dendrocytes and Class II antigen positive cells was greatly increased.

These findings support the suggestion that ALHE is not a tumour, but an immunological reaction against an, as yet, unknown antigen. Possibly the pathogenesis involves a combination of both type IV and type I hypersensitivity reactions.

Nucleolar organizer region associated proteins in benign and malignant cutaneous melanocytic lesions

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The histopathological assessment of malignancy may be difficult in certain melanocytic lesions. It has been shown that the number of demonstrable nucleolar organizer regions (NORs) may reflect the degree of histological malignancy.[1] NORs are portions of ribosomal DNA present in the nucleoli. NORs and NOR-associated proteins may be stained by an agyrophilic technique and visualized as nuclear black granules. We have studied 51 melanocytic lesions comprising 24 benign melanocytic naevi, 12 primary malignant melanomas, nine secondary melanomas and, to date, six dysplastic naevi. Sections were stained with 50% silver nitrate by the method of Crocker and Skilbeck.[1] In each section 100 nuclei were counted by two independent observers.

Benign melanocytic naevi had the fewest nucleolar organizer regions (mean ± SD; 1·43 ± 0·30). Both primary and secondary melanomas had significantly higher mean counts than benign naevi (4·19 ± 1·50, $P < 0{\cdot}001$ and 4·39 ± 1·32, $P < 0{\cdot}001$, respectively; Mann-Whitney U test). There was no significant difference in numbers of NORs between primary and secondary melanoma. There was a significant difference between dysplastic naevi (1·90 ± 0·24) and benign naevi ($P < 0{\cdot}01$) and between dysplastic naevi and primary melanomas ($P < 0{\cdot}001$).

The silver-stained NORs thus clearly distinguish between benign and malignant lesions. As there was a significant difference and no overlap between dysplastic naevi and malignant melanomas, the technique may be useful in differentiation of equivocal melanocytic lesions. This technique clearly has potential in other areas of dermatopathology.

REFERENCE

1 Crocker J, Skilbeck N. Nucleolar organiser region associated proteins in cutaneous melanotic lesions: a quantitative study. *J Clin Pathol* 1987; **40:** 885–9.

Skin and liver

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This presentation deals with several interrelationships between diseases of skin and liver:

(1) Diseases with lesion in both sites (porphyrias). (2) Disease associations (lichen planus and primary biliary cirrhosis). (3) Pathological similarities (interface dermatitis and piecemeal necrosis). (4) Dermal manifestations of liver disease (vasculitis) (5) Hepatic effects of dermatological treatment (methotrexate). (6) Effects of toxins on both skin and liver (arsenic).

Outcome of carbon dioxide laser therapy for persistent viral warts

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It has been suggested that carbon dioxide (CO_2) laser ablation is appropriate therapy for persistent viral warts on the hands and feet.[1] Recently, 22 such patients have been treated at this hospital and we present the long-term results in this group.

Four patients have been lost to follow-up. The remaining 18 (13 females, five males) had a median age of 29 years (range 16–64 years). One patient was immunosuppressed following a renal transplant, the others were healthy. Twelve patients had plantar warts, the other six had warts on the hands (mainly periungual). Treated areas were solitary in eight cases and multiple in 10. The median duration of warts prior to laser treatment was 5 years (range 7 months to 20 years), and all cases had received and failed to respond to other forms of appropriate treatment.

After obtaining informed consent, warts were vapourized under local anaesthesia using a CO_2 laser in defocussed mode. An average of four passes at a power setting of 8–20 W was required. One patient was re-treated 3 weeks later. Wounds were dressed with topical mupirocin (Bactroban®) until re-epithelialization.

After an average follow-up of 10 months (range 6–14 months), all treated warts were cleared in 10 patients (56%), some had cleared in three and in the remaining five patients all the treated warts had relapsed. There was clinical evidence of scarring in 10 patients and this was tender in eight cases. In contrast to previous reports, post-operative pain was universal. In 14 cases this was considered to be severe, lasting for a median of 3 weeks, but up to 9 months in one case. Temporary loss of function of the treated part was common, but in two cases full function had not returned at the time of follow-up (9 and 12 months, respectively). Eight patients considered the treatment to have been worthwhile, i.e. they were better off after the treatment than before, despite the morbidity. However, nine patients were considered to have had a bad result, being worse off after the treatment.

Whereas the CO_2 laser may cure otherwise resistant viral warts, there is significant morbidity with this form of treatment.

REFERENCE

1 McBurney EI, Rosen DA. Carbon dioxide laser treatment of verrucae vulgares. *J Dermatol Surg Oncol* 1984; **10**: 45–8.

The treatment of recalcitrant viral warts with high dose cryosurgery under local anaesthesia

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The aim of the present study was to compare a 'standard' liquid nitrogen spray cryosurgery regimen with higher dose liquid nitrogen application in the treatment of recalcitrant viral warts, particularly in view of the emergence of the use of CO_2 laser and other surgical procedures for which local anaesthesia is required.

Patients of both sexes (aged 17–39 years) with recalcitrant warts (defined as those failing to respond to at least 6 weeks' treatment with standard wart paint and at least three treatments with liquid nitrogen) occurring bilaterally on the hands ($n=16$) or the plantar surface of the feet ($n=15$) were recruited. Following randomization, the warts on one hand or foot received a 'standard' single 10 s liquid nitrogen freeze (hand) or two 15 s freezes (foot), whilst the warts on the other hand or foot received higher dose liquid nitrogen spray under lignocaine local anaesthesia, consisting of two 20 s freezes (hand) or two 30 s freezes (foot). These freeze times represent the duration of continuous liquid nitrogen spray after the ice margin had extended immediately beyond the edge of each lesion. Where two liquid nitrogen freezes were used, the lesion was allowed to thaw before the second freeze.

On review 4 weeks after treatment, 69% (11 of 16) of the hand warts, and 20% (3 of 15) of the plantar warts which had received higher dose liquid nitrogen spray appeared to have cleared, none recurred during follow-up of 2–12 months, and there was no significant scarring. In contrast, none of the lesions treated by the 'standard' regimen had cleared on review at 4 weeks.

Thus, standard liquid nitrogen treatment was completely ineffective in clearing recalcitrant viral warts. This treatment should, therefore, be re-appraised, particularly as only one-fifth of the plantar warts responded to higher dose treatment. Higher dose treatment did, however, clear about two-thirds of recalcitrant hand warts and may be an acceptable alternative to other surgical procedures for such lesions.

Cryotherapy for skin cancer

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A review has been undertaken to assess the efficacy of cryotherapy in the treatment of basal cell carcinomas (BCC) or squamous cell carcinomas (SCC) which were too large for simple primary excision or situated at an anatomically difficult site, e.g. the inner canthus. Five hundred and six tumours were treated by cryotherapy in 405 patients (mean age 70 years). The mean follow-up period was 40 months (range 6–120 months). A recurrence at the treated site occurred in 8·4% of patients. Table 1 shows the histological type and recurrence rate of the treated tumours. A new tumour at a different site developed in 10·9% of patients during the follow-up period.

The recurrence rates for solid and superficial BCC and SCC were similar, but treatment of invasive or morphoeic BCC was less successful. Of the 34 tumours which recurred, 19 (56%) did so within 12 months of treatment. New tumours continued to develop at the same rate each year during the follow-up period.

The data suggest that cryotherapy is a satisfactory form of treatment for tumours which are large or situated in an anatomically difficult site, and that a 12-month follow-up period will enable detection of the majority of recurrences.

TABLE 1.

Histological type	No. treated	No. of recurrences	Recurrence rate (%)	No. of new tumours
BCC solid	306	19	6·2	40
BCC invasive or morphoeic	42	7	16·6	5
BCC superficial	63	4	6·3	10
SCC	95	5	5·2	4

Transmission of virus particles by caustic and styptic pencils and liquid nitrogen containers—a problem for dermatologists?

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Dewar flasks of liquid nitrogen into which swabs potentially contaminated with viruses such as human papillomavirus (HPV), human immunodeficiency virus (HIV) or hepatitis B virus (HBV) are repeatedly dipped, are widely used by dermatologists. Similarly, caustic (silver nitrate) and styptic (aluminium sulphate) pencils are used for a variety of purposes including haemostasis. In the current 'virus conscious' climate, it is vital to know whether these multi-treatment modalities could result in virus transmission from patient to patient.

To answer this question, we have looked at the morphology and infectivity of virus after exposure to aluminium sulphate, silver nitrate and liquid nitrogen. Two viruses which can be safely handled in the laboratory were used, herpes simplex type I (an enveloped virus of interest in its own right and because it has characteristics similar to HIV) and adeno virus 2 (a non-enveloped virus with characteristics similar to HPV and HBV). Morphology was assessed by electron microscopy and viability by the ability to infect Vero cells (Herpes) or HEp-2 cells (adeno).

After exposure to aluminium sulphate or silver nitrate neither virus was able to infect cells in culture (despite retaining a normal morphology in the case of aluminium sulphate. In contrast, after inoculation of Dewar flasks of liquid nitrogen, infective herpes and adeno virus could be recovered after the liquid nitrogen had 'boiled off' and from the 'dry' flask the following day. We suggest, therefore, that swabs should not be dipped repeatedly into the liquid nitrogen and that flasks should be sterilized before refilling.

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SUMMARIES OF POSTERS

(1) Chronic bullous disease of childhood around the world

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Chronic bullous disease of childhood (CBDC) is a rare autoimmune disease characterized clinically by sub-epidermal bullae and annular lesions with a predilection for the perineum. Most published series are from Europe and North America; there are also case reports from Jamaica, Africa, Singapore and Japan. In this co-operative study with South Africa and Sri Lanka (1985–1987) it would seem that CBDC is more common in these countries. During this period 11 patients from South Africa (10 African, one Asian), eight patients from Sri Lanka (all Asian) and three from the U.K. were seen.

In the U.K. and Sri Lanka the populations sampled were very similar in number. However, only data from Durban in South Africa was available. Minor differences in the age at presentation of CBDC were seen and a female predominance was found only in the African series. Linear basement membrane zone staining for IgA was demonstrated on direct immunofluorescence in all patients. In addition, six of 11 African and four of eight Sri Lankan patients had weakly positive staining for IgM. Circulating anti-basement membrane zone antibodies were found in six of the eight Sri Lankan patients and two of the three U.K. patients.

Dermo-epidermal separation by chemical means (1 M NaCl) showed binding of the circulating IgA to the epidermal side in all cases. Immunoelectron microscopy on two African patients showed deposition of antibody in the sub-lamina densa.

These preliminary studies would suggest that CBDC is more common in African and Asian populations than in the U.K. sample. That all patients have an identical disease is supported by the immunopathological findings. However, this may require different environmental factors for its expression.

(2) Erythema induratum: a clinical and histological review

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In 1861, Bazin described a form of panniculitis associated with tuberculosis in young women. This was characterized by recurrent nodules on the lower legs which showed caseation necrosis on histological examination. More recently, the tuberculous aetiology of erythema induratum has been questioned. We report the clinical and histological findings in 31 patients with erythema induratum.

Thirty-one patients were reviewed: 24 were women and seven men, mean age 45 years (range 19–74). They complained of protracted (mean duration 4·7 years, range 1 month–16 years), tender, recurrent nodules, usually on the lower legs (90%), but also on the arms (16%), thighs (16%), feet (6%) and buttocks (3%). A third of the patients had a family or personal history of exposure to tuberculosis.

All patients had a positive Mantoux test: 84% reacting with > 1 cm induration to 1:10,000 Mantoux (1 PPD unit). The ESR was raised (> 25 mm in the first hour) in 8 of 20 patients (40%) and a chest X-ray was abnormal in 3 of 22 cases. Skin biopsies were taken from 22 patients. All but one patient had a deep dermal inflammatory cell infiltrate around vessels. The subcutis showed a lobular panniculitis in all cases, with areas of fat necrosis and foamy histiocytes. Granulomas were absent in over half of the cases, and were for the most part small, non-caseating, poorly circumscribed and composed of epithelioid histiocytes. Ziehl-Neelson stains were negative.

All 31 patients responded to anti-tuberculosis treatment: the skin lesions resolved, the general health of the patients improved, and several gained weight. In all, 24 of 31 were completely cured, three relapsed (none of whom had received adequate therapy), and four were lost to follow-up.

Although it has not been possible to fulfill the required criteria for diagnosing tuberculosis, we believe that there is sufficient evidence to support erythema induratum being a true tuberculide. We would like to emphasize three points: firstly, erythema induratum is not rare and is frequently misdiagnosed; secondly, the Mantoux test solution (1:10,000) must be injected correctly into the dermis and thirdly, patients require 9 months of triple anti-tuberculous therapy.

(3) Calcium enhances the sensitivity of immunofluorescence for pemphigus antibodies

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Indirect immunofluorescence (IIF) to detect pemphigus and pemphigoid autoantibodies is commonly performed with monkey oesophagus (MO) as substrate and phosphate-buffered saline (PBS) as diluent. The purpose of this study was to evaluate comparative IIF titres using human skin (HS) as substrate with variations in the buffers employed. Substrates (MO or HS) were incubated in PBS, Tris-acetate buffered saline (TAS), TAS with 5 mM CaCl^{+2}(TAS-Ca^{+2}) and PBS or TAS with 1 mM ethylenediamine tetraacetate (EDTA), prior to incubation with pemphigus or pemphigoid (BP) sera. In 20 of 21 pemphigus sera, endpoint IIF titres were higest on HS incubated with TAS-Ca^{+2}. Six sera (2PV/4BPF) had end-points which were 5 double dilutions higher, and 2 PV sera were equivalent with both substrates and buffers. Pre-incubation of either substrate with EDTA prior to IIF abolished PV and BPF antibody binding completely. Exposure to EDTA after the tissue was incubated with PV or BPF sera did not affect IIF titres. With the addition of Ca^{+2} to the buffer the pemphigus antigen retained IIF reactivity in the presence of trypsin in concentrations of 0·001%. In the absence of Ca^{+2}, IIF reactivity was destroyed by 0·0001% trypsin. These differences in IIF endpoint titres were not observed with BP sera. This study shows a significant increase in the sensitivity of IIF assays for pemphigus autoantibodies by the use of Ca^{+2} supplemented buffers on HS substrate. This finding may have implications for procedures designed to purify or detect pemphigus antigens.

(4) Sesquiterpene lactone mix: 6 months experience

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Dermatitis from chrysanthemum and other compositae plants is well described, but patch-testing with the plants or oleoresins can cause false negative results or false positive irritant reactions. Ideally, pure allergens extracted from the plants should be used for patch testing. A sesquiterpene lactone mix (0·1% in petrolatum) has recently been developed (Professor C.Benezra).

Initially, the terpene mix was in our light series for 3 months, but as 12 patients were positive we incorporated the mix into the standard series for the next 3 months. In the previous 6 months there had been only one positive out of 82 patients tested with chrysanthemum leaf.

Five hundred and thirteen patients have been tested; 26 (5%) showed positive, allergic, elicitation reactions. A further two patients were actively sensitized with a 1% mix. In 17 patients (eight occupational) the reactions were of current significance, in four the relevance was not known, in four there was presumed exposure, but no history of dermatitis from plants and in one a past history of chrysanthemum dermatitis. Only three out of 16 patients, additionally tested with chrysanthemum leaf, were positive to the leaf. In seven patients chronic actinic dermatitis had been suspected.

Sesquiterpene lactone mix is extremely useful in the investigation of suspected compositae and photosensitive dermatitis, and it is suggested that the mix (0·1%) be in the standard series, as less than half of the 17 current relevance patients were suspected before patch-testing.

(5) Genital warts and cervical intraepithelial neoplasia—a 10-year follow-up in a dermatology unit

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A high prevalence of both cervical infection by the human papilloma virus (HPV) (50%) and cervical intraepithelial neoplasia (CIN) (36%) has been reported in women attending a sexually transmitted disease clinic.

We have performed a retrospective study of all women seen by the Dermatology Department in the last 10 years with genital warts in order to determine the outcome and follow-up to institute. There have been 110 referrals, and the annual average has not significantly changed.

Seventy-three per cent of patients have had cervical smears since diagnosis, of which 6% showed CIN.

Of those women presenting before 1980, there have been no cervical abnormalities recorded to date.

One patient who had a normal smear 3 years after presentation with perianal warts developed grade 2 CIN after 6 years. Despite having, since 1984, advised annual cervical smears for our patients with genital warts, of the 14 women who presented in 1985 only four have had their first follow-up smear of which two have shown changes of CIN.

Women who attend dermatology clinics with genital warts are at risk of CIN which may occur as long as 6 years after diagnosis. The recall system is inadequate and in future it may be necessary for the dermatologist to follow-up these patients.

REFERENCE

1 Walker *et al.* Abnormalities of the uterine cervix in women with vulval warts. *Br J Venerol Dis* 1983; 59: 120–3.

(6) Investigation of the swollen limb with isotope lymphography

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Chronic lower limb oedema is a common medical problem confronting many clinicians including dermatologists. Most cases arise from a disturbance in the vascular mechanisms controlling homeostasis in the limb. Three main clinical categories of chronic limb oedema may arise depending on the extent of lymphatic involvement: lymphoedema—from an obstruction to normal lymph flow with no venous abnormality; lymphostasis—'armchair legs' in circumstances of orthostasis and immobility where there is a normal or slightly increased lymph load; lymphovenous disease—where chronic venous insufficiency places extra demands on lymphatic drainage by considerably increasing the lymph load.

We report our experience in patients with lower limb oedema and the value of isotope lymphography for assessing lymphatic insufficiency. The technique employs the subcutaneous injection of ^{99m}Tc-antimony colloid (Nanocoll, Solco-Nuclear) simultaneously into the first web spaces of both feet. Detection is performed over the feet, legs and inguinal regions using a large field of view gamma camera at intervals of up to 2 h post injection. The speed of clearance of the tracer and uptake in the regional nodes equates with lymphatic function. Of 13 patients investigated, isotope lymphography established impaired lymphatic function in 10, while three patients exhibited enhanced lymph drainage associated with venous disease.

Until now radiocontrast lymphangiography has been the only investigation for lymphoedema. Isotope lymphography is a non-invasive functional test and is now the investigation of choice for chronic oedema of primarily lymphatic origin.

(7) A new method for the quantification of physical change in unstable pigmented lesions using digital image processing techniques

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The incidence of malignant melanoma is rising rapidly and early detection significantly reduces the mortality rate. Some acquired and congenital melanocytic naevi may be important risk factors for this malignancy. Accurate photographic documentation is important for all pigmented lesions and is part of the recommended surveillance in the management of patients with dysplastic naevi.[1,2] Conventional photography provides information on the simple parameters of minimum and maximum diameters. The new technique of digital image analysis allows several other parameters to be recorded and stored. These measurements include intensity of erythema and pigmentation, lesion perimetry and surface area. The parameters, when measured routinely over a period, should provide a very accurate assessment of change and possibly detect earlier progression to malignant melanoma.

REFERENCES

1 MacKie RM, English J, Ashworth J, Doherty V. Managing the dysplastic naevus. *Lancet* 1983; **i:** 1249–50.
2 Atkinson JM, From L, Boyer R. A new method of photodocumentation for the follow-up of dysplastic naevus. *J Audiov Media* 1987; **10:** 12–14

(8) The routine use of keratin monoclonal antibodies in the differential diagnosis of cutaneous amyloid

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Clinicopathological identification and classification of cutaneous amyloidoses may be difficult in some cases. This study demonstrates the value of monoclonal antibodies to cytokeratins in determining the possible histogenesis of various types of cutaneous amyloidosis.

The low molecular weight anti-cytokeratin antibody CAM 5·2 identifies simple epithelium and LP 34 is a pan-cytokeratin marker which detects all epithelial structures in frozen tissues. These monoclonal antibodies were compared with conventional amyloid staining methods on routine formalin-fixed paraffin sections.

Indirect monoclonal immunoperoxidase and routine staining techniques were used to examine 18 skin biopsies. These included biopsies from 12 patients with lichen amyloidosus, one nodular amyloidosis, two myeloma-associated renal amyloidosis and three basal cell epitheliomas with amyloid degeneration.

Amyloid deposits were labelled with LP 34 in 83% (10 of 12) of the biopsies from lichen amyloidosus and all three basal cell epitheliomas. Nodular and primary systemic amyloid did not react with LP 34. CAM 5·2 was negative in all cases. Congo red and thioflavine T staining confirmed the presence of amyloid in all specimens examined.

Our results support previous suggestions that the degenerating keratinocytes provide the matrix for the deposition of amyloid in lichen amyloidosus.[1,2] In our study LP 34 gave reliable labelling of amyloid deposits in routine paraffin sections comparable with conventional staining methods. This study demonstrates that the histogenesis of nodular and systemic amyloidosis differs from that of lichen amyloidosus and secondary cutaneous amyloidosis. The use of LP 34 on routine paraffin sections in the diagnosis of cutaneous amyloidosis may influence the clinical management of these conditions.

REFERENCES

1 Black MM, Wilson Jones E. Macular amyloidosis. A study of 21 cases with special reference to the role of the epidermis in its histogenesis. *Br J Dermatol* 1971; **84:** 199–209.

2 Kobayashi H, Hashimoto K. Amyloidogenesis in organ-limited cutaneous amyloidosis: an antigenic identity between epidermal keratin and skin amyloid. *J Invest Dermatol* 1983; **80:** 66–72.

(9) Tumour necrosis factor and psoriasis

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Tumour necrosis factor (TNF)α is a cytokine with multiple biological properties, particularly pro-inflammatory, apart from the induction of tumour necrosis. Significant serum levels can be found in 50% of cancer patients, but only 3% of normals. Serum TNFα levels have been measured by ELISA in the serum of members of a family with five affected members with severe psoriasis and in culture supernatants of cultured psoriatic fibroblasts and keratinocytes, and DNA from cultured fibroblasts and peripheral blood lymphocytes has been probed by Southern blotting against a TNF gene probe. Elevated serum levels of TNFα have been found (two-thirds), and culture supernatants from psoriatic fibroblasts and keratinocytes has shown evidence of TNFα activity, but no genomic polymorphism. This suggests that TNFα may have a role in inflammation in psoriasis and it may be locally produced in the skin. Further studies on larger populations are in progress.

(10) Energy-dispersive X-ray microanalysis (EDX): a method to assess the elemental composition of hair

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Scanning electron microscopy is frequently applied to dermatological problems. Quantitative analysis in the electron microscope by use of EDX is proving an important accessory technique. From an analytical point of view, an electron microscope may be used as an analytical system incorporating an X-ray tube.

Plucked, untreated scalp hairs are mounted onto aluminium stubs with double-sided cellotape and then carbon coated. They are scanned at a tilt of 45° and analysed at 20 kn for 100 s (live time) using an Hitchi H-3010 electron microscope fitted with an energy dispersive detector coupled to a Link Systems Ltd. 860 Series 1 Multichannel analyser.

Spectra are collected at the root, above the root, 0·5 cm above the root, midshaft and tip of the hairs, and processed using a Link Systems Ltd. ZAF/PB programme. The elements considered are sulphur, calcium, chlorine, potassium, sodium, phosphorus, silica, zinc, iron and copper. The results are expressed as percentages of the total spectrum minus the background.

Spectra recorded from normal hairs show that the major element detected is sulphur which is constant along the hair shaft. There is a linear increase in calcium levels from root to tip. The roots of anagen hairs contain more sulphur and potassium than telogen roots.

We have described a potentially useful method of assessing the elemental composition of hair.

(11) Langerhans cell histiocytosis: is this a malignant process?

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Although there has been much recent controversy, Langerhans cell histiocytosis (LCH), or histiocytosis X, is now generally considered to be a non-malignant condition. A flow cytometric study of a single case has, however, been published which claimed to provide evidence to contradict this. The presence of DNA aneuploidy as detected using this technique is a feature of malignant and pre-malignant disease. In this reported single case, DNA aneuploidy was present, although the patient was atypical of LCH in many ways.[1] We report a study of six cases of LCH, five of whom had multisystem disease. In addition, in one of these, fresh tissue studies using an anti-CD1 monoclonal antibody were performed.

Sections of formalin fixed, paraffin embedded tissue 60 μm thick were processed for flow cytometry as described previously.[2] The DNA was stained using DAPI. A further fresh tissue sample was processed for DNA flow cytometry both with and without labelling with anti-CD1 (OK T6) antibody in order to specifically examine the LCH cells.

All six patients (nine samples) had entirely normal, diploid DNA profiles. The fresh tissue sample had clear labelling with the anti-CD1 antibody of 4% of the total number of cells. Furthermore, these CD1 +ve cells were also diploid.

To date there has been only a single reported DNA profile of a patient with LCH. We report a study of six cases using both fixed and fresh tissue incorporating anti-CD1 labelling specifically to examine the LCH cells. In all cases, the DNA content of cells was entirely normal. We, therefore, have found no evidence that LCH is a neoplastic process.

REFERENCES

1 Goldberg NS, Bauer K, Rosen ST *et al.* Histiocytosis X. *Arch Dermatol* **122:** 446–50.
2 Newton JA, Camplejohn RS, McGibbon DH. A flow cytometric study of the significance of DNA aneuploidy in cutaneous lesions. *Br J Dermatol* 1987; **117:** 169–74.

(12) Incidence of seborrhoeic dermatitis in spinal injury patients

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Many anecdotal reports state that seborrhoeic dermatitis is more common in immobile patients. The Spinal Injury Unit at Stoke Mandeville Hospital specializes in the care of patients immobilized due to cervical cord injury. We undertook a prospective study to observe the incidence and temporal relationship of seborrhoeic dermatitis to spinal injury. Twenty newly injured patients were examined on admission and then regularly for 4 months. No patient had clinically significant seborrhoeic dermatitis on admission, or had a past history of it; six patients did report dandruff in the past. During the study 13 patients (65%) developed typical seborrhoeic dermatitis mainly on the face and upper chest. All of these developed the eruption within 7 weeks of injury and 10 patients within the 1st month.

The remarkably high incidence of seborrhoeic dermatitis in this group of patients is probably due to factors in addition to obligatory recumbency. It cannot be due to sebum excretion rate alone as this has been shown to be increased below the level of the cord lesion.[1] Two observations may be of particular relevance. As a result of the need to keep spinal movement to a minimum following a recent injury, hair is not washed and facial cleansing is by gentle bed bathing only. The greasy nature of the patient's unwashed hair and the possible accumulation of sebum and scale on inadequately scrubbed skin, could promote the overgrowth of *Pityrosporum ovale*.

REFERENCE

1 Thomas SE, Conway J, Ebling FJG, Harrington CI. Measurement of sebum excretion rate and skin temperature above and below the neurological lesion in paraplegic patients. *Br J Dermatol* 1985; **112:** 569–73.

(13) The use of the carbon dioxide laser in dermatology

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One hundred and twenty-four patients have received 161 treatments with the continuous wave carbon dioxide laser during the past 3 years. Fifty-one patients had port wine stains (PWS) and 48 had tattoos. The remaining patients had a variety of ectasias, hypertrophies and tumours.

The 51 patients with PWS can be divided into two groups—those under 16 years of age (22 patients, mean age 10·1 years, range 1–15 years) with predominantly pink, easily compressible naevi, and adults who had failed to respond to either Argon or Dye laser therapy (29 patients, mean age 33·1 years, range 16–61 years). Seventy treatments were given, 57 under general anaesthesia and 13 with local anaesthesia. A Coherent 451 carbon dioxide laser with a dermatological handpiece was used. Power levels between 4 and 8 W with a spot diameter of 5 mm were used in continuous mode.

Follow-up results 9 months or more after therapy are available on 38 patients (16 children, 22 adults). The laser produced an excellent or good response in 24 (63%), fair in eight, and poor in two (5%)—one with a hypertrophic scar and one with marginal hyperpigmentation. No effect was observed in four patients. These results are extremely promising as both groups responded poorly to Argon laser therapy. Pallor is slow to develop and improved responses with longer follow-up are expected. Six-month follow-up after carbon dioxide laser therapy in 31 patients with tattoos is available. Treatment was with 5–10 W in defocussed mode and then 5–10 W with spot size of 0·2 mm and pulse duration of 0·05 s (energy fluence 6–12 J/cm^2) to residual pigment. Three patients failed to attend follow-up. The remaining 28 patients were pleased with the result and wanted further therapy for other tattoos. Objectively, all patients developed a scar, which was hypertrophic in two. Two patients had residual pigmentation, one post-inflammatory hyperpigmentation, and three required antibiotics for wound infections.

Other lesions treated have included plantar warts (5), keloids (4), facial telangiectases (4), lymphangioma circumscriptum (3), verrucous haemangiomas (2), and trichoepitheliomata (2), all with satisfactory results.

In conclusion, the carbon dioxide laser is the most versatile laser for the treatment of a wide variety of dermatological conditions and appears particularly useful in pink port wine stains, if used cautiously, and in the treatment of tattoos where patients will accept scarring.

(14) A comparison of the efficacy and relapse rates of 311 nm phototherapy with etretinate 311 therapy (re-311) and etretinate PUVA (re-PUVA) in psoriasis therapy

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Etretinate combination therapies using UVB (re-UVB) and PUVA (re-PUVA) are well established in the management of severe psoriasis, enabling clearance with an average of one-third of the total irradiation required with UVB or PUVA alone. While the use of broad-band UVB lamps is limited by burning, a new '311' lamp has been developed,[1] with narrow-band emission at 311 nm (±2 nm), producing less burning[2] and less potential carcinogenesis. This study compares 311 UV monotherapy with etretinate plus 311 (re-311) and etretinate plus PUVA (re-PUVA), in groups of 15 patients with extensive psoriasis. All had previously either failed to respond to UVB or PUVA, rapidly relapsed following clearing, or had had a high cumulative UVA PUVA dose (>1000 J/cm^2). Results in 38 patients showed that nine of 12 in the 311 group achieved a satisfactory response in 4·6 weeks (13 treatments) with a total exposure dose of 11·38 J/cm^2 (all values means); 13 of 15 patients in the re-311 group had a satisfactory response in 4·3 weeks (10 treatments) and a significantly lower total exposure dose of 6·73 J/cm^2. In the re-PUVA group all 11 patients achieved a satisfactory response in 7·5 weeks (11 treatments) with an exposure dose of 42·2 J/cm^2. To date, a superior remission has been achieved with re-PUVA; seven of 11 patients were in remission for more than 5 months, compared with five of 12 on 311 and three of 15 on re-311. Thus, all three therapies were effective in producing a remission of severe psoriasis, the duration of which was best in the re-PUVA group.

REFERENCES

1 Van der Leun JC *et al.* UVB phototherapy; principles, radiation sources, regimens. *Curr Prob Dermatol* 1986; 15: 39.
2 Johnson BE *et al.* Ultraviolet radiation phototherapy for psoriasis. the use of a new narrow band UVB fluorescent lamp. *Photochem Photobiol* 1987; 1 (Suppl. 1): 49.

(15) Elastic fibre damage with low dose pencillamine—a review of 18 patients with rheumatoid arthritis

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High dose penicillamine treatment may produce cutaneous effects including skin fragility, elastosis perforans serpiginosa and pseudoxanthoma elasticum (PXE)-like changes. The elastic fibres in these lesions show a characteristic 'bramble-bush' contour.

A rheumatoid arthritis (RA) patient with widespread cutaneous and systemic penicillamine induced elastosis has been described.[1]

Since penicillamine induced elastosis was previously unreported at doses used to treat RA, the skin and joints of 18 RA patients receiving penicillamine were studied. Elastic tissue was examined in 14 biopsies of non sun-exposed skin, six surgical joint specimens and 15 control specimens from RA patients not treated with penicillamine.

Six patients reported the onset of skin fragility during penicillamine treatment. Yellowish papules were found on the necks of two patients. PXE-like change was present on the neck of one patient.

Biopsies from non-exposed skin did not show 'bramble-bush' elastosis, but this feature was present in the biopsy from skin with PXE-like change and in four of the six joint specimens. No such change was found in the 15 control specimens.

This study demonstrates that penicillamine may cause elastic tissue damage in both skin and joints at doses used to treat RA. Factors influencing individual susceptibility to penicillamine elastosis remain unclear.

REFERENCE

1 Burge S, Ryan T. Penicillamine induced pseudo-pseudoxanthoma elasticum in a patient with rheumatoid arthritis. In: *The CMD Case Collection* (Wilkinson DS, Mascaro JM, Orfanos CE, eds) Stuttgart: Schattauer, 1987; 325–6.

(16) Investigation of a low magnesium dialysate in uraemic pruritus

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Renal itch affects up to 86% of patients on chronic haemodialysis and the therapeutic options are severely restricted. The aim of the study was to evaluate the role of a low magnesium dialysate in the treatment of uraemic pruritus, in view of the isolated reports of benefit from this therapy,[1] and our recent finding of a positive correlation of renal itch with serum magnesium concentration in chronic renal failure.

Seventeen patients with chronic renal failure on maintenance haemodialysis and known to suffer with uraemic pruritus, were recruited into the study. The patients were randomized blind into two groups to receive dialysis against either a magnesium free dialysate, or a standard solution containing 1 mmol/l magnesium. After 2 weeks the patients were crossed over to receive the alternative dialysate for a further 2 weeks. The effect of altering the dialysate was monitored by weekly measurement of serum magnesium and potential interaction with parathyroid hormone (PTH) secretion checked by fortnightly assay of PTH. Itch was assessed throughout the 4 week trial by the patients completing visual analogue scales.

Hypermagnesaemia on the standard dialysate was significantly lowered after 2 weeks treatment with the magnesium free dialysate, but no significant alteration in either the PTH level, or the amount of uraemic pruritus was demonstrated.

Objective investigation has failed to corroborate previous suggestions that a low magnesium dialysate is effective in uraemic pruritus.

REFERENCE

1 Graf H, Kovarik J, Stummvoll HS, Wolf A. Disappearance of uraemic pruritus after lowering dialysate magnesium concentration. *Br Med J* 1979; ii: 1478–9.

(17) Interferon versus bleomycin in the treatment of severe persistent viral warts—preliminary results

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The aim of this study is to compare the effect of intralesional injections of human lymphoblastoid interferon-α (Wellferon®) with bleomycin in the treatment of severe, persistent, cutaneous, viral warts failing to respond to the vigorous application of conventional therapy.

Informed, consenting, adult patients were entered into a fully randomized, parallel, open trial and were scheduled to receive either Wellferon® (3 mega units) or bleomycin (0·3 mg) injected intralesionally into their largest or most troublesome wart at weekly intervals for up to 12 weeks. Patients were followed up for 3 months after cessation of treatment. Careful clinical assessments, supported by a photographic record, were made at regular intervals throughout the study period on both injected and non-injected lesions.

It is intended to complete studies on 60 patients. Data from 17 patients (nine male, eight female; age range 16–53 years; mean age 30·9 years) whose warts had been present for at least 1 year (range 1–17 years; mean 6·1 years) are currently available for analysis. Seven out of nine lesions treated with Wellferon® were completely cleared and two showed non-significant (< 50%) clearance. Five out of eight lesions treated with bleomycin were completely cleared with two additional warts showing significant (> 50%) clearance. Of the patients with distant non-injected warts, three out of six treated with Wellferon® showed complete (2) or significant (1) clearance and two out of six treated with bleomycin demonstrated significant, but not complete, clearance.

These preliminary results suggest that human lymphoblastoid interferon-α is at least as effective as bleomycin in the treatment of severe cutaneous viral warts.

(18) The contribution of direct immunofluorescence (DIF) to the diagnosis of lupus erythematosus

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To investigate the contributions of serology, light microscopy and DIF to the diagnosis of lupus erythematosus (LE) we performed an audit of 55 biopsies submitted between 1973 and 1985, selected on the basis of availability of long-term follow-up data. The contribution of each investigation to the final diagnosis was assessed as unhelpful, against, consistent with or highly suggestive. DIF was assessed as highly suggestive of chronic discoid lupus erythematosus (CDLE) if IgG, IgM and IgA were present at the dermo-epidermal junction and consistent with CDLE if one or a combination of IgA, IgM and C_3 was present. For systemic lupus erythematosus (SLE) DIF was assessed as highly suggestive if IgM or IgG or a combination of both was present in both lesional and non-lesional skin. Serology was said to be highly suggestive of SLE if there was raised DNA binding or an ANA of titre $\geqslant 1:64$ plus decreased C_3 or C_4 and consistent with SLE if an ANA of titre $\geqslant 1:64$ or a decreased level of C_3 and C_4 were present alone. In the 55 biopsies, DIF was found to be highly suggestive of the final diagnosis in nine cases (16·5%) and consistent with the final diagnosis in 17 cases (31%). When subdivided into initial clinical suspicion of possible CDLE, possible SLE and 'please exclude' LE, DIF was found to be most useful in contribution in the possible SLE group. Nine investigations out of the 24 suspected cases (37·5%) were assessed as highly suggestive of SLE. In only three of these nine cases was serology also highly suggestive of SLE although in another three cases it was incomplete. In the investigation of possible CDLE and requests to exclude LE, DIF was consistent with the final diagnosis in 12 out of 24 (50%) and four out of 24 (16%), respectively, but was highly suggestive of the final diagnosis in no cases (0%). The most useful investigation was light microscopy.

DIF was most useful in the diagnosis of SLE when a diagnosis of CDLE or a request to exclude LE was made, DIF should not be performed until the results of light microscopy and serology are known.

(19) Which emollients should be used during phototherapy (UVB)/ photochemotherapy (PUVA) for psoriasis? A study of emollient effect on minimal erythema (MED) and phototoxic (MPD) doses

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While some emollients are known to enhance phototherapeutic effectiveness in psoriasis[1] by a reduction in reflectance within plaque scale, others such as Red. Vet. Petrolatum are known to be sunscreens. Precise guidance on emollient choice is lacking. Because a psoriatic patient failed to clear while using yellow soft paraffin (YSP) as a pre-treatment before phototherapy, but responded well when changed to coconut oil, we studied the effect of selected emollients on MED and MPD values. Thirty-five patients commencing phototherapy (15) or photochemotherapy (20) were studied using YSP, white soft paraffin (WSP), 50% liquid paraffin/50% WSP (50/50) or coconut oil. These were applied to separate 14 × 4 cm areas of back skin at 4·5 mg/cm^2 (within the known self-application range); each area of application was irradiated with a range of six doses of UVB (Phillips TL 40W/01) or PUVA (Sylvania UVA). The absorption spectrum of each agent (6 μm thickness) was obtained using a Pye Unicam spectrophotometer SP8000.

Results indicated that YSP and WSP-treated areas had significantly elevated MED and MPD values when compared with untreated control sites ($P < 0·01$), in some cases the MED being raised by a factor of 4. While 50/50 increased MED values, coconut oil affected neither MED nor MPD (Table 1). Absorption spectroscopy supported these results. We conclude that WSP and YSP should be avoided during UVB or PUVA therapy. In our study coconut oil appears suitable as a pre-treatment emollient.

TABLE 1.

	Control	YSP	WSP	50/50	Coconut oil
MED mean mJ/cm^2	353	1167	764	611	390
n	15	9	9	10	10
MPD mean J/cm^2	2 0	4 1	2 7	1 8	2 1
n	20	8	8	9	8

REFERENCE

1 Levene MJ *et al.* Components of the Goeckerman regimen. *J Invest Dermatol* 1979; 73: 170–3.

(20) Topical retinoic acid in the treatment of solar elastotic degeneration

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The long-term effects of chronic exposure to ultraviolet radiation include alteration in the dermal collagen (solar elastosis). Animal studies have suggested that prolonged application of topical retinoic acid may reverse some UVR-induced connective tissue damage by stimulating collagen synthesis or by inhibition of collagenase.

We have studied the clinical and histological effects of topical retinoic acid in patients with solar elastosis in a double-blind, placebo-controlled paired comparison. Twenty patients with solar elastotic degeneration of the face or forearms and backs of the hands or both were recruited. Patients applied 0·05% retinoic acid cream to the study area on one side and a placebo cream to the other for 3 months, with assessment each month. Clinical evaluation of the severity of clinical signs was recorded on separate visual analogue scales including wrinkling and discolouration.

There was a significant difference between treatments ($P < 0·01$). Of the 17 patients who completed the study, 14 were judged to have improved by global assessment on the side treated with retinoic acid, two on the placebo treated side, and one did not improve at all. Skin stiffness measurements, using a uniaxial linear extensometer did not show consistent changes, but using the pulsed ultrasound technique there was a statistically significant increase in total skin thickness in retinoic acid treated sites compared with placebo treated sites ($P < 0{\cdot}001$). Punch biopsy specimens (4 mm) were taken from the treatment sites before and after treatment. However, no reduction in the degree of solar elastosis was apparent in the visual analogue scale assessment used. The alteration in clinical appearance and thickening in the treated sites certainly suggests that dermal changes take place after treatment. The anomalous histological results could indicate insensitivity of the method used or be the result of sampling errors.

(21) Cytokine mRNA in psoriatic skin detected by *in situ* hybridization

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The cytokines interleukin 1 (IL1) and tumour necrosis factor (TNF) have been implicated in the pathogenesis of inflammatory and immune diseases. Resident skin cells (keratinocytes and Langerhans cells) as well as infiltrating mononuclear cells, can all produce IL1 when activated *in vitro*. IL1 peptides have been identified in extracts of human psoriatic skin and also in suction-blister fluid.

IL1 gene expression is largely controlled at transcription, and activated cells contain IL1 mRNA. We wondered whether cells activated for IL1 gene expression could be identified in psoriatic skin samples. Biopsies of psoriatic plaques were snap frozen in liquid nitrogen and cytokine mRNA content was tested for by slot analysis. Thin (8 μm) sections were fixed on microscope slides. *In situ* hybridization of specific mRNAs was performed using ^{32}P-labelled cDNA for human IL1 beta and TNF alpha. Irrelevant DNA sequences were used as negative controls.

Results showed cells containing IL1 beta mRNA in both the epidermis and dermis of psoriatic lesions. The TNF alpha probe hybridized mainly with dermal cells. We are now immunophenotyping these sections with monoclonal antibodies to identify the cell types that contain these transcripts. Our results demonstrate that cells in both the epidermis and dermis are activated for cytokine gene expression in the inflammatory lesion itself during the course of the disease.

(22) Plasma exchange in refractory cutaneous vasculitis

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Patients with severe cutaneous vasculitis may fail to respond to conventional immunosuppressive therapy. We have studied the effects of plasma exchange in nine patients (five females, four males; age range 31–65) with leukocytoclastic vasculitis and predominant cutaneous involvement, who had previously not responded to combinations of corticosteroids, azathioprine cyclophosphamide and melphalan.

Circulating immune complexes were detected in sera from seven patients prior to plasma exchange using either a C_{1q} binding assay or a rheumatoid factor binding assay. One patient had a 'benign' IgA paraprotein. Significant levels of cryoglobulins (>200 μg/l) were not detected and only one patient had hypocomplementaemia.

Initial treatment on an out-patient basis consisted of three 4 l plasma exchanges for 5% human albumin. A clinical assessment was made within 24 h of the exchange and at 1 or 2 weekly intervals thereafter. Five patients showed a dramatic clinical improvement within hours of plasma exchange; one patient was subsequently unable to continue therapy because of difficulties with vascular access, but the other four patients have been controlled solely by regular plasma exchanges for 5–12 years, receiving one to three 4 l exchanges every 3–6 weeks via an arteriovenous fistula. The procedure is well tolerated and has resulted in no adverse sequelae apart from one episode of hepatitis B, possibly related to the administration of fresh frozen plasma.

This study indicates that some patients with a severe refractory cutaneous vasculitis can be successfully managed as out-patients with plasma exchange.

(23) Junctional epidermolysis bullosa: a clinicopathological review of 30 patients

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Recent classifications include as many as six distinct forms of junctional epidermolysis bullosa (JEB) thereby stressing the heterogeneity of the disorder, but complicating efforts to elucidate the pathogenesis and devise effective treatment. We have reviewed our findings in 30 cases of JEB examined over the past 9 years using a conventional clinicopathological approach recently augmented by studies with specific anti-basement membrane antibodies. The diagnosis in all patients was confirmed by electron microscopy. Twenty-one patients (12 males, nine females) are included in the Herlitz (lethal) group. Blisters were invariably present at or within 1 week of birth and tended to be widespread involving any part of the skin and often the mouth. Persistent facial erosions were found in all four patients surviving 1 year or longer. Sixteen patients died within 6 months; three died aged 2 to 5 years; two are still alive aged 2 months and 3 years, respectively. Indirect immunofluorescence using antibodies AA3 or GB3 or both universally showed reduced or absent staining of the epidermal basement membrane. Six patients aged 25 to 65 years (four females, two males) are included in the non-lethal group. All have atrophic lesions with a generally fragile skin. One has 'inverse' JEB. Three have sparse scalp and body hair and three have severe dental abnormalities. One developed a squamous cell carcinoma in the atrophic pretibial skin. Pruritus was present in four patients. GB3 or AA3 staining or both was normal in three patients and reduced in three patients. Hemidesmosomes were normal in two patients. We encountered no patients with 'cicatricial' or 'progressive' JEB. Three patients aged 2 to 7 years remain unclassified. There was no consistent correlation between immunohistochemical and ultrastructural findings. We conclude that JEB is heterogeneous, but a definitive classification must depend on better biochemical and genetic information. Recent antibody studies may provide the best lead yet to discovering a common defect among these groups of patients.

(24) Experience of sulphamethoxypyridazine in the treatment of sub-epidermal bullous dermatoses

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One hundred and three cases of dermatitis herpetiformis were reviewed to compare the clinical response to and incidence of side-effects of dapsone and sulphamethoxypyridazine. Thirty patients received sulphamethoxypyridazine (0·5–1·5 g/day) as a single agent therapy at some stage during their care. Eighty-five received dapsone (25–400 mg/day) as a single agent treatment. Twelve of these patients had received both agents, but at different times.

Both drugs were highly effective in controlling the skin disease. Only four (13%) patients on sulphamethoxypyridazine and two (2%) on dapsone changed to different drug treatment because of ineffective therapy. However, whereas only two (6·5%) changed from sulphamethoxypyridazine because of intolerable side-effects, 16 (19%) of the dapsone treated group changed for this reason. The major side-effects are shown in Table 1.

TABLE 1.

	Sulphamethoxy-pyridazine ($n=30$)	Dapsone ($n=85$)
Anaemia	1 (3%)	11 (13%)
Neuropathy/paraesthesia	0	5 (6%)
Hypersensitivity rash	1 (3%)	0
Severe headache	0	3 (3 5%)
Nausea necessitating drug withdrawal	0	3 (3 5%)
Lethargy necessitating drug withdrawal	0	2 (2%)

Sulphamethoxypyridazine was also found to be helpful in the management of linear IgA disease, and the oral and cutaneous lesions of cicatricial pemphigoid.

Sulphamethoxypyridazine is a useful drug in the management of subepidermal bullous dermatoses. It should probably be the first line drug therapy in the elderly, and for those who are unable to tolerate the side-effects of dapsone.

(25) Long-term follow-up of low-dose cyclosporin treatment for psoriasis

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Thirteen patients with chronic severe psoriasis not responsive to other current therapeutic modalities were treated with long-term, low-dose, cyclosporin. The mean duration of treatment was 18·2 months (range 10–26 months). Clinical response was assessed by PASI scores (psoriasis area and severity index, range 0–72). All subjects improved. The PASI scores were reduced from a mean of 23·2 (range 8·4–51·0) before treatment to 7·3 (range 0–23·4) at 8 weeks, and a mean score of 5·1 (range 1·8–9·6) on long-term follow-up. The dose of cyclosporin ranged from 1 to 4 mg/kg/day. There was no correlation between cyclosporin blood levels and clinical response. Five patients had a significant rise in blood pressure; in two cases this was transient and in three cases continuing antihypertensive treatment was necessary. The serum creatinine level did not rise above the upper limit of the normal range in any of the patients. Four patients developed hypertrichosis, and two developed seborrhoeic eczema.

Cyclosporin is a highly effective treatment for severe, intractable psoriasis, and is an alternative to other forms of systemic treatment.

(26) Cutting oil dermatitis

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Skin contact with cutting oils, especially soluble oils used in industry, is a major cause of occupational dermatitis. Although soluble oil dermatitis is often irritant, sensitization may occur to one or more components of soluble oils, which are complex water-soluble mixtures of lubricants, emulsifiers, biocides, extreme pressure additives and antifoam agents. We have reviewed 174 patients, presenting to two contact dermatitis clinics over 2 years between 1986 and 1987, in whom cutting oil dermatitis was suspected.

The dermatitis was classified as allergic, irritant, endogenous, or a combination, on the basis of clinical assessment and patch tests, including a standard series and a cutting oil series. Irritant contact dermatitis occurred in 63% of the patients, but only in 21% was it thought to be the sole diagnosis. Forty-one per cent of patients showed positive allergic reactions which were thought to be relevant to their dermatitis; 20% were allergic only, 18% were both irritant and allergic and 3% allergic, irritant and endogenous. Formaldehyde-releasing biocides were the commonest cause of allergic reactions in the cutting oil series. Reactions were also seen to colophony, formaldehyde, epoxy resin, rubber, dichromate and nickel.

As nearly half the patients had relevant positive patch-tests, patch-testing remains an essential investigation in the management of cutting oil dermatitis.

(27) Port wine stains: treatment with argon laser or infra-red coagulator?

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The Argon laser (AL) is well established as a treatment for port wine stains (PWS), but the newer infra-red coagulator (IRC) which is cheaper, portable, quicker and easier to use, has been suggested as producing comparable results. The purpose of this study was to compare the cosmetic results on the same PWS using the AL and IRC.

The IRK 151 delivers a spectrum of radiation with a maximum ouput of 960 nm in the infra-red range. The pulse duration is preset between 0·7 and 0·9 s[1] and infra-red energy converted to heat in the dermis with relative epidermal sparing. The AL was a Coherent 9007 with a 1 mm beam diameter used at minimal blanching power plus 0·05 W.

Ten patients with cutaneous PWS had adjacent test areas treated by each machine under local anaesthesia. They were reviewed monthly and then at 3 monthly intervals for 18 months. Both methods resulted in lightening of the PWS. In 6 of 10 of the AL-treated areas there was no epidermal change. However, there were considerable complications from the IRC; 4 of 10 patients developed hypertrophic scars (none with the AL), 4 of 10 post inflammatory hyperpigmentation (none with AL), 2 of 10 developed depressed scars and 2 of 10 epidermal atrophy (1 of 10 developed each of these with AL). These were most marked at highest pulse durations. Nodular plaque-like PWS paled and flattened well with the IRC. The optimal pulse duration was shorter (0·6–0·75 s) than previously recommended.[1] Minimal alteration in pulse duration made a considerable difference between no cosmetic improvement and the complications which will be illustrated.

We conclude that the IRC must be used cautiously and is not a tool for widespread untrained use, that the IRC is best reserved for nodular and plaque-like PWS and that the AL remains the treatment of choice for macular PWS.

REFERENCE

1 Colver GB, Cherry GW, Dawber RPR, Ryan TJ. The treatment of cutaneous vascular lesions with the infra-red coagulator: a preliminary report. *Br J Plastic Surg* 1986; **39**: 131–5.

INVITED POSTERS

(28) Dermatology at Guy's Hospital: the first hundred years

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One hundred and twenty-five years after the founding of Guy's Hospital, the first separate Skin Out-patient Department opened in 1850. That year, Thomas Addison was appointed the first 'Demonstrator of Cutaneous Diseases', in addition to his other duties as a general physician. Although most famous for his descriptions of adrenal disease and pernicious anaemia, he contributed two original descriptions of dermatological disease, namely those of xanthomata and keloid.

In the mid-nineteenth century, Guy's Hospital was fortunate to have on the staff Joseph Towne, perhaps the greatest of all wax modellers. Not only were his models extremely accurate, but he was also a prolific and tireless worker. At the instigation of Thomas Addison, over 300 wax models of dermatological disease were produced and classified according to the system of Robert Willan. Much of this collection still exists in Guy's Hospital Medical School today.

Over the next 70 years the Skin Department was staffed by a succession of eminent Victorian physicians of Guy's Hospital. These included Sir William Gull, Sir Samuel Wilks, who described verruca necrogenica and striae atrophicae as well as a vast literature on syphilis, Charles Hilton Fagge, who reclassified all the wax models, and Sir Philip Pye-Smith who had studied abroad with Virchow in Berlin and Hebra in Vienna.

In 1919, upon the retirement of Sir Edwin Cooper Perry, whose fame as an able hospital administrator extended to Africa, Harold Barker was appointed the first full time 'Physician to the Skin Department' with no other duties. He had studied widely in Europe with eminent dermatologists such as Darier, Gougerot and Unna. Geoffrey Dowling wrote that Barber 'dominated thought in British Dermatology'. His retirement in 1949 marked the end of a century of dermatology at Guy's.

(29) Cutaneous Reactions Database—Committee on Safety of Medicines Joint Collaborative Project

A.KOBZA BLACK, M.W.GREAVES AND A.I.MALLET

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Cutaneous reactions to drugs are poorly reported by non dermatologists. Therefore, a specialist based system is necessary to obtain a true picture of their type and incidence. Skin reactions often provide warning of more toxic side effects of a new drug. Unexpected beneficial cutaneous effects of drugs can be reported and the information disseminated. Funds have been obtained to create such a database for a trial period of 2 years. It will be a collaborative project with the Committee on Safety of Medicines (CSM). Consultants, senior registrars and clinical assistants in dermatology in the British Isles will be issued with special reporting forms which can be mailed to the Cutaneous Reactions Database (CRD—CSM), sited at the Institute of Dermatology, St. Thomas' Hospital.

Alternatively, the report may be telephoned by a direct line to the CRD—CSM, where it will be recorded by a trained person. Two written copies will be sent to the reporter for verification, and one corrected version returned to the Institute by Freepost. For these reactions, there will be no need to fill in a yellow CSM card, as the information requested on it will be relayed to the CSM. The CRD—CSM project will enjoy identical protection to that of the CSM yellow card. Follow-up of cutaneous reactions will be undertaken by the CRD—CSM from the Institute, and early warnings of adverse side effects mailed to doctors in collaboration with the CSM. Information on adverse reactions due to drugs previously reported to the CSM and the CRD—CSM, will be made available on request. A data terminal from the Adverse Reaction Databank of the CSM will be available at the Institute. We will undertake to provide regular update reviews of the cutaneous effects of drugs, for publication in the dermatological literature. The aim of the CRD—CSM is to be a centre for collating and disseminating information in a service that will benefit patients and dermatologists.

British Journal of Dermatology (1988) **118**, Supplement **33**, 61–118.

CASE REPORTS

(1) Granuloma faciale

DRS M.HAMILTON AND I.SARKANY

M.G., female, aged 34 years.

History. This 34-year-old lady presented in 1986 to another hospital with two soft, slowly enlarging red-brown plaques on the left upper cheek. These lesions were asymptomatic and there were no associated systemic symptoms such as weight loss, night sweats or malaise. Examination at that time revealed two red-brown lesions 2 cm × 1 cm. These were excised, but no firm histological diagnosis was made. However, the density of the infiltrate was such that the possibility of a cutaneous lymphoma was raised.

A year later she presented to the Royal Free Hospital with a recurrence of the lesions. These were small, soft, red-brown domed plaques with a slight prominence of the hair follicles. The appearances were thought to be suggestive of granuloma faciale. Clinical examination was, otherwise, normal.

Investigations. Full blood count and blood film, urea and electrolytes and liver function tests were normal. Chest X-ray and abdominal CT scan showed no lymphadenopathy, and a bone marrow aspirate was normal.

Histology of one of the lesions showed the following features. The upper dermis contained a pleomorphic cellular infiltrate composed of lymphocytes, plasma cells, eosinophils and many polymorphs. Many small dermal blood vessels were damaged and showed evidence of an associated leukocytoclastic vasculitis. The epidermis was uninvolved and a 'Grenz' zone separated the epidermis and adnexal structures from the main infiltrate. These appearances are typical of granuloma faciale.

Comment. Granuloma faciale is a chronic condition primarily involving the face and characterized by one or more red-brown nodules or plaques. The clinical differential diagnosis of granuloma faciale includes, cutaneous lymphomata, discoid lupus erythematosus, angiolupoid sarcoidosis and leishmaniasis.

The lesions are benign, but tend to persist. Although intralesional triamcinolone is partially effective, surgical excision may eventually be required.

REFERENCES

1 Schnitzler L, Verret JL, Schubert B. Granuloma faciale. Ultrastructural study of three cases. *J Cut Pathol* 1977; **4**: 123–33.

2 Rusin LJ, Dubin HV, Taylor WB. Disseminated granuloma faciale. *Arch Dermatol* 1976; **112**: 1575–77.

(2) Cockayne's syndrome

DRS G.M.MURPHY, P. G.NORRIS AND J.L.M.HAWK

St John's Hospital for Diseases of the Skin, London

J.A., female, aged 14 years.

History. This patient was born of non-consanguineous parents and has a normal sister. She had a normal delivery, but was hypoxic and required resuscitation after birth. Early development was unremarkable, but she was slow to stand and walk and was noticeably ataxic by 2 years of age. Investigations at that time revealed shortness of stature and microcephaly. She had mild amblyopia of the right eye, fine central retinal pigmentation, pale optic discs and partial hearing loss (50–60 db). She had an intention tremor, brisk tendon reflexes and mental retardation. At 4 years of age she developed a recurrent photosensitive erythematous papular eruption which occurs 24 h after sun exposure, lasts 2 weeks, settles with itching and desquamation, and is now controlled with topical sunscreens.

Since then her hearing and vision have gradually diminished, and she requires a wheelchair because of increasing ataxia. Menarche occurred at 9 and normal secondary sexual characteristics are present.

Now at 14 she has a mental age of 4–5 years, and her condition appears reasonably stable. She can dress herself and attends a special school.

Examination. She is mentally retarded, short in stature, has microcephaly with deep set eyes and prominent supra-orbital ridges, and her limbs are disproportionately long. She can only walk short distances because of ataxia.

Investigations. X-rays of the wrist and digits showed dysharmonious skeletal maturation. X-rays of the skull revealed microcephaly; there was no intracranial calcification. A CT scan showed moderate enlargement of the third and fourth ventricles with widening of the basal subarachnoid space, consistent with brain stem atrophy. Monochromatic light tests showed normal responses at 24 h, but exaggerated responses with normal minimal erythema doses at 300–320 nm at 48 h. The sister chromatid exchange rate was normal. Cultured fibroblasts from a skin biopsy showed normal excision and post replication repair. Fibroblast survival following UVC irradiation was impaired. Inhibition of DNA and RNA synthesis following UV irradiation of cultured fibroblasts failed to recover.

Comment. Cockayne's syndrome is a rare autosomal recessive disorder. This patient illustrates most of the typical findings. The diagnosis has been substantiated by demonstrating increased sensitivity of cultured fibroblasts to UV irradiation and impaired RNA and DNA synthesis following inhibition by UV irradiation, in the presence of normal DNA repair and sister chromatid exchange.

ACKNOWLEDGMENT

We are grateful to Dr M.Dakar for measurements of sister chromatid exchange and Dr F.Giannelli for the cultured fibroblast studies.

REFERENCES

1 Cockayne EA. Dwarfism with retinal atrophy and deafness. *Arch Dis Childhood* 1936; ii: 1–8.

2 Mayne LV, Lehmann AR. Failure of RNA synthesis to recover after UV irradiation: an early defect in cells from individuals with Cockayne's syndrome and xeroderma pigmentosum. *Cancer Res* 1982; 42: 1473–8.

(3) Twenty-nail dystrophy of childhood associated with alopecia areata and lichen planus

DRS D.A.FENTON AND P.D.SAMMAN

St. John's Hospital for Diseases of the Skin, London

M.D., male, aged 11 years.

History. All fingernails and toenails have been thin and rough since 2 years of age. For 4 years he has had patchy hair loss involving his scalp. He developed an itchy rash on his arms and abdomen 1 year ago.

An identical twin is unaffected.

Examination. All nails show trachyonychia, with ridging and thinning of the nailplate without scarring, consistent with twenty-nail dystrophy of childhood. He has patchy alopecia areata affecting the occiput and crown. There are widespread micropapules over the wrists, forearms and abdomen.

Investigations. Scalp histology shows alopecia areata, and a skin biopsy from the wrist shows lichen nitidus.

Comment. Twenty-nail dystrophy is a self-limiting disorder usually occurring in childhood and clearing spontaneously in the early teens. The aetiology has been linked to both alopecia areata and lichen planus (although scarring almost never occurs). This unusual case is associated with both conditions; but in view of the lack of scarring of the nails and spontaneous improvement, alopecia areata would appear to be a more likely cause.

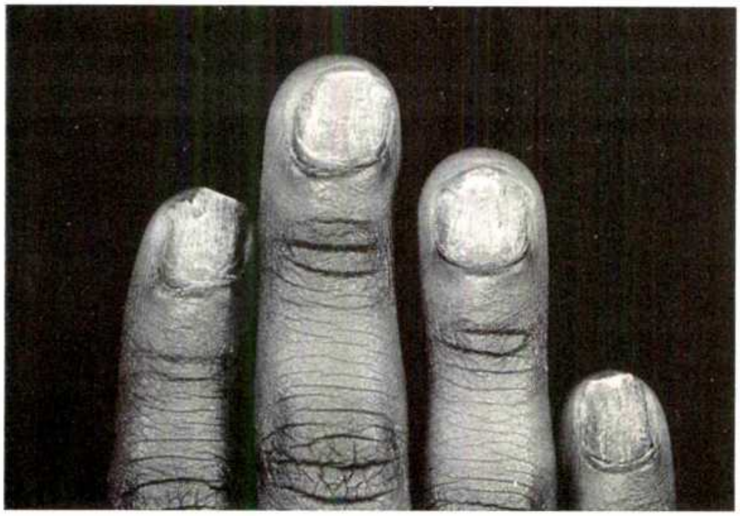

FIGURE 1. Twenty-nail dystrophy.

REFERENCE

1 Hazelrigg DE, Duncan C, Jarrett M. Twenty-nail dystrophy of childhood. *Arch Dermatol* 1977; **113:** 73–5.

(4) Lichen planus in a father and son

DRS A.MILLIGAN AND R.GRAHAM-BROWN

Leicester Royal Infirmary

M.A., male, aged 42 years, with son, S.A., aged 10 years.

History. Patient M.A. has an 18-year history of lichen planus which began at the age of 24 years, with lesions on the limbs. These were noted to be follicular and hypertrophic. Remission occurred with intralesional triamcinolone, but hypertrophic lesions on the feet and ankles recurred subsequently.

Patient S.A., his son, has a 2-year history of lesions on the limbs and trunk.

Examination. M.A. has lesions of hypertrophic lichen planus on the ankles and feet.

S.A. has lesions of follicular lichen planus, some of which are in a linear distribution. There is also post-inflammatory pigmentation and stria formation (? secondary to topical steroid therapy).

Investigation. A skin biopsy in patient M.A. confirmed lichen planus.

Treatment. M.A. is responding to treatment with potent topical steroids and Haelan tape. His son's skin lesions have only partially responded to topical steroid therapy.

Comment. Familial lichen planus and lichen planus in children are both uncommon. Where the latter occurs it is often not classical in its appearance and is resistant to therapy, as in this case.

(5) Lichen planus pemphigoides

DRS M.E.MURDOCH AND I.M.LEIGH

The London Hospital, Whitechapel, London

W.T., male, aged 74 years.

History. This gentleman has a 40-year history of hypertrophic lichen planus of the shins treated by repeated excision and grafting. He presented in February 1987 with a long-standing, enlarging, hyperkeratotic fissured area at the lower end of the left shin scar. Initial biopsy at this site showed changes only of lichen planus but a repeat biopsy performed in November 1987 revealed a well-differentiated squamous cell carcinoma. He, therefore, underwent wide excision of the tumour with split skin grafting.

Five days after the operation he developed a pruritic patchy erythematous eruption on the hands, followed by an erythematous papular rash on the right leg with subsequent vesicle formation. Reactivation of his lichen planus, possibly triggered by the stress of his operation was postulated and he was treated with topical steroids. Two weeks later, however, he had progressed to an urticated erythematous eruption on the trunk and lower limbs with moderate-sized tense bullae on the legs suggestive of bullous pemphigoid.

He was commenced on oral prednisolone 30 mg daily with rapid clearing of the blisters. The steroid dose was gradually reduced and azathioprine added and he has remained blister-free since.

Examination. (December 1987) He had a blistering eruption with moderate-sized tense bullae mainly on the lower legs, arising both on areas of urticated erythema and on normal-appearing skin.

Long-standing findings include a violaceous plaque on the right shin and nail dystrophy. He has never had oral involvement.

Skin biopsy (urticated erythematous lesion on thigh). Routine histology: the superifical dermis is infiltrated by eosinophils and mononuclear inflammatory cells, showing perivascular accentuation. There is oedema and degeneration beneath the basal layer, but no fully formed bullae. Appearances suggest early pemphigoid.

Immunohistology (direct immunofluorescence and immunoperoxidase). Linear BMZ fluorescence is seen with IgG and C_3, consistent with bullous pemphigoid.

Indirect immunofluorescence. Weak patchy staining along the BMZ with a 1 : 10 dilution of the patient's serum with IgG anti-serum. This patchy reaction is not typical of pemphigoid.

Treatment. The bullous eruption was rapidly controlled by prednisolone 30 mg daily. With the addition of azathioprine 100 mg daily, the steroid dose was successfully reduced over 4 weeks to 10 mg daily.

Comment. Our patient fulfilled the criteria for lichen planus pemphigoides, namely typical lichen planus (clinically and pathologically) in association with a bullous eruption on uninvolved skin. Furthermore, the bullae arose particularly on the extremities with pathological and immunological features of bullous pemphigoid, including *in vivo* bound and circulating anti-BMZ antibodies. There has, however, been uncertainty as to whether lichen planus pemphigoides should be regarded as an association between lichen planus and bullous pemphigoid or a separate disease entity.

In 1983 a case of lichen planus pemphigoides was described[1] in which it was proven by immunoprecipitation that the circulating antibodies were not directed against the bullous pemphigoid antigen. Preliminary Western blotting results using our patient's serum similarity suggest that his circulating antibodies are not directed against the bullous pemphigoid antigen. Furthermore, immuno-electron microscopy[2] demonstrated that immunoreactants were present along the floor of a bulla of lichen planus pemphigoides and not along the roof as occurs in bullous pemphigoid. Our patient's immuno-electron microscopy results will be discussed. It seems likely that the circulating autoantibody in lichen planus pemphigoides is recognizing an antigen distinct from the bullous pemphigoid antigen.

REFERENCES

1 Lang PG, Maize JC. Coexisting lichen planus and bullous pemphigoid or lichen planus pemphigoides? *J Am Acad Dermatol* 1983; **9:** 133–40.

2 Prost C, Tesserand F, Laroche L. *et al.* Lichen planus pemphigoides: an immuno-electron microscopic study. *Br J Dermatol* 1985; **113:** 31–6.

(6) Type VII Ehlers Danlos syndrome

DRS R.LEUNG AND R.RUSSELL JONES

Ealing Hospital, London

T.M., male, aged 46 years.

History. Fragile skin has been noted since birth. The patient has suffered from recurrent dislocation of both thumbs, premature dental caries, leg ulceration and painful plantar callosities. An umbilical hernia was repaired in early childhood. There is no positive family history.

Examination. The patient is of short stature and has severe kyphoscoliosis and multiple pigmented scars over the cheeks, forehead and chin. Pseudotumours are present over the elbows and knees, and extensive ecchymoses over the shins, knees, elbows and knuckles. The skin is hyper-extensible with gross joint laxity and prominent epicanthic folds. There is no evidence of cardiac or ocular abnormalities.

Investigations. X-rays showed marked kyphoscoliosis of the lumbar spine with associated degenerative changes, bilateral Medelung ulnar abnormalities and discrete foci of calcification in the soft tissues.

Comments. This patient's clinical phenotype most closely resembles Ehlers Danlos syndrome type VII, but this awaits biochemical confirmation. He was reported in the paediatric literature in 1947 when aged 4. Prominent inner canthus, scarring of the nose and forehead, hyper-elastic skin, joint hyper-extensibility, atrophic scarring of knees and multiple bruises were clearly evident at that time. In the absence of significant cardiovascular involvement, his life expectancy is normal.

REFERENCES

1 King-Lewis FL, Polunin IV. Two cases of Ehlers-Danlos syndrome. *Arch Dis Child* 1947; **III**: 170–1.

2 Steinmann B, Tuderman L, Peltonen L. *et al.* Evidence for a structural mutation of procollagen type I in a patient with the Ehlers-Danlos syndrome type VII. *J Biol Chem* 1980; **255**: 8887–93.

(7) Ehlers Danlos syndrome type IV presenting with swollen legs

DRS C.M.PROBY, P.S.MORTIMER AND R.A.MARSDEN

St. George's Hospital, London

P.C., female, aged 41 years.

History. The patient was small at birth, weighing 3 lbs. In early childhood progressive joint deformities affected her hands, especially. Subsequently, she has had numerous operations to improve finger contractures, including silastic replacement of interphalangeal joints. An atrioseptal defect, sinus deformities and right congenital corneal opacity were all noted during childhood. She has been troubled by extensive varicosities of the superficial veins of both legs since puberty with serious haemorrhage from a venous varicosity at the age of 18 years.

Swelling of both legs, present for many years and attributed to lymphoedema, has been progressive, with recurrent episodes of cellulitis.

Family history. Her father and sister have both had severe varicose veins from a young age. There is no history of lymphoedema.

Examination. Unusual facies with a pointed nose, lobeless ears, blue sclerae and premature arcus. The skin over her extremities is soft and atrophic. Prominent veins are visible over her shoulders, chest and forearms. There are joint deformities with marked contractures of the interphalangeal joints in both hands. There is gross, asymmetrical, chronic oedema of both legs (the right more than the left) with some pitting.

Investigations. A chest X-ray showed an enlarged main pulmonary artery.

A hand X-ray showed fixed flexion deformities of PIP and DIP joints. Venograms of both legs were normal. Isotope lymphography demonstrated good lymphatic function from feet to inguinal nodes bilaterally. Skin collagen studies were carried out by Dr M.Pope, Northwick

Park Hospital, London. Type III collagen was deficient in cultured fibroblasts. This is diagnostic of Ehlers Danlos syndrome type IV.

Comment. Ehlers Danlos syndrome type IV (EDS IV) is a distinctive syndrome in which acrogeria, characteristic facial appearance and joint deformities are combined with lethal or life-threatening vascular weakness. It results from defective type III collagen. Both autosomal dominant and autosomal recessive inheritance are recognized.

This patient has many of the features of EDS IV, but she presented most unusually with grossly swollen legs. Isotope lymphography disproved a diagnosis of lymphoedema and suggested that the swelling was due to widespread incompetence of the venous system resulting from defective collagen support of the vasculature.

(8) Bloom's syndrome

DR B.MONK

Bedford General Hospital

P.F., male, aged 14 years.

History. This child was noted at birth to have odd, elfin facies, and has since shown progressive growth retardation; his intelligence, however, is normal. Marked photosensitivity became apparent from the age of 6 months, sun exposure producing erythematous plaques over the cheeks, forehead, and ears, and the backs of the hands. These lesions resolve to leave linear, depressed scars. His photosensitivity is controlled by meticulous use of sunscreens.

Family history. His parents and siblings are unaffected, but his paternal grandfather is said to have died of acute myelomonocytic leukaemia at the age of 47.

Investigations. Sister chromatid exchanges, measured by the Clinical Genetics Unit of Guy's Hospital in 1977, showed 110 per cell (control value, 9 per cell).

Histology. A skin biopsy of an active lesion at the age of 2 years shows striking hydropic degeneration of the basal layer.

Comment. This child shows the characteristic features of Bloom's syndrome. Such children have a high incidence of leukaemia and lymphoma, and the family history of leukaemia may not be fortuitous, perhaps representing partial expression of a genetic trait.

(9) Hereditary multiple fibrofolliculomas: Syndrome of Birt, Hogg and Dubé

DRS R.K.CURLEY, P.S.MORTIMER AND R.A.MARSDEN

St. George's Hospital, London

Y.B., female, aged 21 years.

History. This lady had numerous small, white, dome-shaped papules at the sites of previous acne. Her mother has similar lesions on the upper back, and numerous skin tags around the neck.

Examination. Multiple small white papules are present on the anterior chest, shoulders, and back. Keloid scarring is present on the left shoulder.

Histology. A biopsy of a papule showed a circumscribed fibrous nodule with an altered pattern of elastic staining suggestive of a trichodiscoma.

Comment. In 1977 Birt, Hogg, and Dubé[1] reported a kindred of 70 individuals, 15 of whom had multiple, small, 2 to 4 mm dome-shaped skin tumours. The lesions involved the forehead, face, neck, chest and back. Biopsies from clinically identical lesions showed fibrofolliculoma or trichodiscoma. In addition, affected patients developed numerous ski intermingled with the other lesions and involving the upper eyelids, axillary fold antecubital fossae. All three types of lesion are hamartomas, with a specific fibromatous re pattern. Inheritance is thought to be autosomal dominant.

REFERENCE

1 Birt AR, Hogg GR, Dubé WJ. Hereditary multiple fibrofolliculomas with trichodiscomas and acrochordo *Dermatol* 1977; **113:** 1674–7.

(10) Pyoderma gangrenosum and IgA paraproteinaemia—response to pl exchange

DRS S.J.WHITTAKER AND A.C.CHU

Hammersmith Hospital, London

C.T., male, aged 65 years.

History. This patient has a 7-year history of a recurrent pustular eruption which is asso with haemorrhagic ulceration over the lower limbs. In 1976 he had mycoplasma pneu which was complicated by cold agglutinin disease and acute renal failure.

Examination. Extensive atrophic scarring over the trunk and limbs with large cribrifor over the left leg.

Investigations. FBC and biochemical profile were normal. Cold agglutinins were detect titre of only 1:8 at 4°C. Protein electrophoresis revealed an IgA kappa paraproteinaemi g/l). There was no Bence Jones proteinuria and a skeletal survey was normal. A bone m aspirate revealed a slight increase in mature plasma cells. No cryoglobulins were detected. of neutrophil chemotaxis and migration were normal.

Histology. A skin biopsy revealed extensive intradermal abscess formation with necr overlying epidermis. There was no evidence of vasculitis. The changes were consistent clinical diagnosis of pyoderma gangrenosum.

Treatment. Prednisolone, azathioprine, minocycline, dapsone and clofazamine were a unsuccessfully in this patient. Pulse therapy with melphalan produced a severe neutropen a series of six plasma exchanges resulted in rapid resolution of the patient's pyoderma.

Comment. This patient's severe pyoderma gangrenosum and associated IgA kappa pa teinaemia failed to respond to a variety of therapeutic agents, but plasmapheresis has re the paraprotein load and produced a dramatic clinical remission.

REFERENCE

1 Clayton R. Pyoderma gangrenosum with cellular immunity defect and red cell aplasia. *Proc Roy Soc Med* 1 571–2.

(11) Juvenile pyoderma gangrenosum

DRS S.C.MAYOU AND D.H.MCGIBBON

St Thomas's Hospital, London

S.F., female, aged 18 years.

History. Since the age of 2 this West Indian girl has had an extensive scarring bullous eruption. Individual bullae are 2–3 cm in diameter and rupture to form large, painful, superficial ulcers up to 30 cm in diameter. Healing occurs slowly with scarring. Involvement is extensive with sparing of the hands and feet. The ulcers only respond to high doses of oral steroids, and for many years she has been on 15–60 mg prednisolone daily. There is no family history of bullous disease.

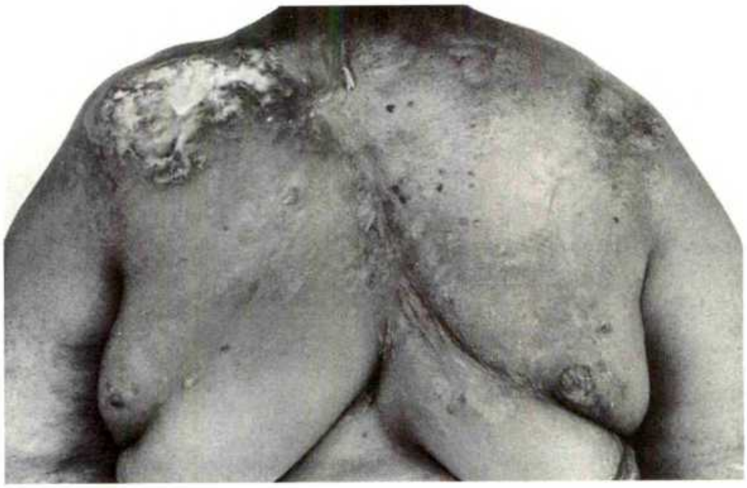

FIGURE 1. A 12 cm diameter superficial ulcer on the left shoulder and severe scarring in juvenile pyoderma gangrenosum.

Histology in 1972, 1977 and 1985 demonstrated sub-epidermal blisters with a dense neutrophilic dermal infiltrate and scarring, but was not diagnostic. Immunofluorescence while on steroids was negative.

Examination. On admission in June 1987 she was obese (110 kg) and Cushingoid. There was a 12 cm diameter superficial ulcer over the left shoulder (Fig. 1), extensive atrophic scarring over trunk and limbs with numerous exuberant warts. The prednisolone was reduced from 60 mg o.d. to 30 mg o.d. to induce new blisters for histological diagnosis.

Investigations. Normal full blood picture, biochemical profile, serum proteins, protein electrophoresis, immunoglobulins, serum zinc and CXR. DNA probe of a single wart showed no HPV 3 or 5. Histology demonstrated an intra-epidermal blister and a suppurative abscess in the mid-dermis and subcutis with eosinophilic necrosis of sweat glands. Gram stain and PAS were negative. Immunofluorescence was negative. The changes are consistent with pyoderma gangrenosum.

Treatment. She has previously received prednisolone 15–60 mg o.d. for 10 years with long courses of dapsone (maximum 200 mg o.d.) and azathioprine (maximum 200 mg o.d.). She is currently reasonably well controlled on prednisolone 45 mg alternating with 35 mg o.d. and minocycline 50 mg o.d.

Comment. Juvenile pyoderma gangrenosum is rare and in only 4% of a recent series of 180 patients with pyoderma gangrenosum had the disease started before the age of 15 years. All of these had underlying inflammatory bowel disease and the cutaneous disease resolved over a mean of 2½ years. Our patient with her chronic resistant pyoderma gangrenosum is proving to be a severe management problem.

REFERENCE

1 Powell FC, Perry HO. Pyoderma gangrenosum in childhood. *Arch Dermatol* 1984; **120:** 757–61.

(12) Scleroderma and polymyositis overlap syndrome associated with IgG lambda paraproteinaemia and cutaneous amyloid deposition

DRS C.B.BUNKER, P.D.L.MAURICE, J.C.SHARP*, A.W.P.DU VIVIER* AND PAULINE M.DOWD

*University College and Middlesex School of Medicine, The Middlesex Hospital, London and *Kings College Hospital, London*

K.S., male, aged 57 years.

History. This man was first seen in 1986 with a 6-year history of severe Raynaud's phenomenon (which had been treated with plasmapheresis in 1980), a 1-year history of generalized weakness and myalgia and 6 months of progressive thickening and tightening of the skin of the fingers, forearms, feet, calves, and face. A benign IgG lambda paraproteinaemia was discovered in 1982. A diagnosis of systemic sclerosis and myositis was made and he was treated with prednisolone (maximum 40 mg daily), azathioprine (maximum 100 mg daily) and oral antibiotics. The muscle weakness has progressively worsened. He has since developed red, scaly patches on his knuckles, elbows and knees.

Examination. There is sclerodactyly with finger tip scars and loss of digital pulp. The nail folds are abnormal with periungual erythema, telangiectatic vessels and raggedness of the cuticles. The scleroderma extends to involve the forearms, calves and lower thighs. There are erythematous scaly patches on the knuckles, elbows and knees. The facies are sclerodermatous and Cushingoid and there is periorbital erythema. The gait is abnormal and there is pronounced proximal wasting and weakness. There is hepatomegaly and there are bilateral basal crackles audible at both lung bases.

Investigations. Relevant abnormal investigations have been: IgG lambda paraprotein with immunoparesis (paraprotein, 8·7 g/l, IgG, 5·15 g/l (normal 8–17·6), IgA, 0·13 g/l (normal 0·65–2·8), IgM, 1·63 g/l (normal 0·9–4·5)); no Bence Jones protein; bone marrow, 11% plasma cells, normal cellularity; triglycerides 2·8 mmol/l (normal up to 1·8); ANF, 1:10–1:40 with an unidentified extractable nuclear antibody; reduced transfer factor on formal testing of pulmonary function; creatine kinase 196–756 IU/ml (normal 24–195); muscle strength testing reduced for his body weight; an electromyogram showed the characteristic abnormalities of myositis (spontaneous fibrillations, early recruitment and polyphasic motor units) from the deltoid only, and a thigh muscle biopsy was equivocal.

Histology. Finger skin biopsy shows the changes of scleroderma. Amyloid deposits are present in the papillary dermis.

Comment. The association between cutaneous amyloid deposition and scleroderma is a rare one and was first described by Black in 1971.[1]

REFERENCE

1 Black MM. Primary localised cutaneous amyloidosis in systemic sclerosis. *Trans St John Hosp Derm Soc* 1971; 57: 177–80.

(13) Giant cell arteritis and systemic lupus erythematosus

DRS C.B.BUNKER AND PAULINE M.DOWD

University College and Middlesex School of Medicine, The Middlesex Hospital, London

J.D., female, aged 63 years.

History. This woman presented in the summer of 1986 with upper limb girdle pain and stiffness, weakness, intermittent claudication, resurgent Raynaud's phenomenon, pallor, absent peripheral pulses, carotid and femoral bruits, livedo reticularis, anaemia (Hb 9·3 g/dl) and a grossly elevated erythrocyte sedimentation rate (ESR) of 130 mm/h. Angiography and digital subtraction angiography suggested a widespread arteritis. A temporal artery biopsy confirmed the diagnosis of giant cell arteritis (GCA). Although antinuclear factor (ANF) was negative and the lupus anticoagulant was absent, the presence of cold agglutinins and anticardiolipin antibodies and a positive 'lupus band test' suggested the possibility of evolving systemic lupus erythematosus. She was treated with oral prednisolone with clinical improvement. During a sunny holiday in 1987 she developed a rash on the forearms and hands and arthralgia.

Examination. A symmetrical eruption of erythematous, hyperkeratotic, raised patches is present on the dorsal surfaces of the fingers, the radial knuckles of the hands and on the forearms. There is livedo reticularis of the lower limbs. Carotid bruits are present.

Investigations. Leukopenia ($2{\cdot}9 \times 10^6$/l), normal differential count; Hb, platelet count, and ESR normal. Antinuclear antibodies are negative. Lupus anticoagulant was not detected although low levels of IgM anti-cardiolipin antibodies persist.

Histology. Histology of affected skin shows hyperkeratosis and parakeratosis with a chronic inflammatory infiltrate in the dermis, most marked in a periadnexal distribution. Immunofluorescence of involved skin shows granular deposition of IgM and C_3 at the basement membrane zone. Immunofluorescence of uninvolved, light protected skin shows linear deposition of IgM at the basement membrane zone.

Comment. There are sufficient clinical and laboratory criteria to allow a diagnosis of systemic lupus erythematosus. The rash has responded poorly to potent and very potent topical steroids and a factor 15 sunscreen, in addition to the systemic therapy listed above. When the arthralgia developed she was started on hydroxychloroquine, but compliance has been poor.

Claudication, Raynaud's phenomenon and bruits may be presenting features of GCA. This patient's Hb and ESR normalized before active treatment with steroids. A normal ESR has been found at the time of diagnosis of GCA in some previously reported cases. An association between GCA and SLE has never appeared in the literature. The prognostic significance of the early presence of livedo reticularis and anticardiolipin antibodies in SLE is the subject of recent interest.

REFERENCE

1 Sontheimer RD. The anticardiolipin syndrome: a new way to slice an old pie or a new pie to slice? *Arch Dermatol* 1987; **123**: 590–5.

(14) Lupus erythematosus

DRS G.DOOTSON AND I.SARKANY

A.B., female, aged 27 years.

History. This secretary has a 7-year history of discomfort over the soles of her feet, which is exacerbated by exercise. More recently, she developed a diffuse erythematous eruption on exposed sites while on holiday in Italy.

Examination. Purple, atrophic skin which is most marked on the weight bearing areas of the sole and dorsum of the forefoot.

Histology. A biopsy from the sole suggested a diagnosis of lupus erythematosus, with widespread liquefaction degeneration of the basal layer and a moderate inflammatory cell infiltrate at the tips of the rete ridges and around superficial dermal vessels. Material for immunofluorescence was unsuitable for analysis.

Investigations. She has a normal haematological profile, and renal and hepatic function. Erythrocyte sedimentation rate was 16 mm in the 1st h. Antinuclear antibody was positive at a titre of 1:80, double stranded DNA titres fluctuated around the upper limit of normal and antibody to SSA (Ro) was positive (+ + +).

Treatment. She has responded well to combined therapy with fluocinonide 0·05% and sodium fucidate.

Comment. This is an unusual presentation of lupus erythematosus. She does not, as yet, satisfy the accepted criteria for a diagnosis of systemic lupus erythematosus, but she should probably be included in the subset of subacute cutaneous lupus erythematosus, in which there is a 60% prevalence of antibody to SSA (Ro) antigen. We have another, exactly similar, patient under our care,[1] who had morphologically identical changes on her feet for a number of years and eventually developed typical lupus erythematous lesions on her face. The clinical appearances of the skin changes on the feet, and sometimes even the histological features may be difficult to differentiate from lichen planus.

REFERENCE

1 Burns DA, Sarkany I. Penicillamine induced discoid lupus erythematosus. *Clin Exp Dermatol* 1979; **4**: 389–92.

(15) Hypertrophic lupus erythematosus

DRS D.C.BUCK, H.J.DODD AND I.SARKANY

N.G., female, aged 29 years.

History. This young negro woman has a 5-year history of systemic lupus erythematosus (SLE), which started with a steroid responsive nephrotic syndrome due to minimal change glomerulo-nephritis. She developed severe lupus nephritis following pregnancy. Despite

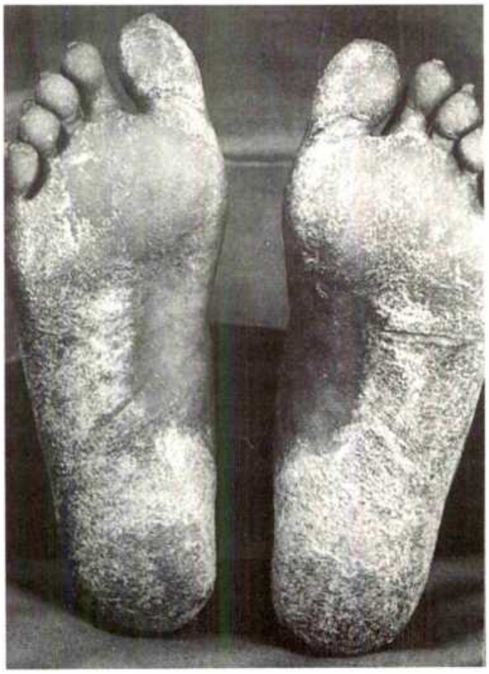

FIGURE 1. Hyperkeratosis of the soles in lupus erythematosus.

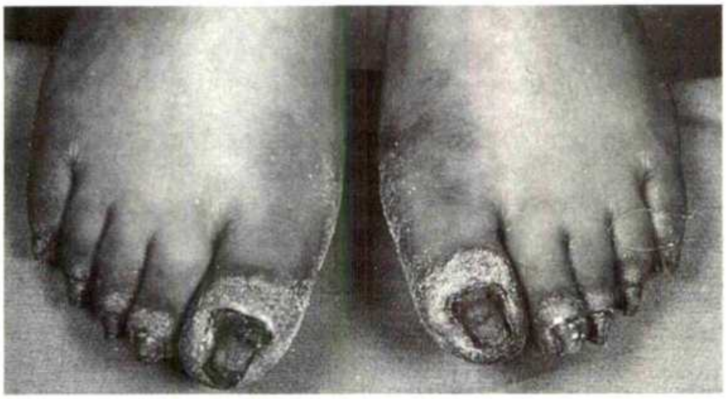

FIGURE 2. Hyperkeratosis affecting the toes in lupus erythematosus.

treatment, this culminated in end-stage renal failure requiring dialysis. In addition to LE positive serology, there was evidence of LE pathology in the lymph nodes, and malabsorption, which also responded to steroids. Her liver function was abnormal, but hepatic histology was non-specific. In 1986 she developed a widespread, florid rash, with large, dark, slightly raised plaques with superficial scale. She has developed progressive hyperkeratosis, generalized ichthyosis and pruritus.

Family history. Her mother died of renal failure in 1968.

Examination. The patient is thin, wasted, anaemic and Cushingoid, with thinning hair and hyperkeratosis of the scalp. She has a hyperpigmented, discoid, scaly plaque near her right eye. Ichthyosis is widespread, especially over the extensor surfaces. In areas where there is less scale, follicular plugging is evident. Gross hyperkeratosis of the palms and soles (Fig. 1) extends onto the dorsa of the toes to surround the nails (Fig. 2). The nails are longitudinally ridged with subungual hyperkeratosis. Warty hyperkeratotic excrescences are present on the knees and elbows.

Investigations. Haematological and biochemical findings are typical of chronic renal failure. A renal biopsy showed crescentic glomerulo-nephritis with deposits of IgM and C_3 in the mesangial area of the glomerular basement membrane. Electron microscopy showed typical SLE deposits.

In the skin biopsy of a warty lesion there was extensive parakeratosis and focal 'psoriasiform' thickening of the epidermis. There was prominent thickening of the basement membrane and mild focal vacuolar degeneration of the basal layers. The superficial dermis included a moderate perivascular mononuclear cell infiltrate and there was some oedema of the papillary dermis with scattered melanophages. Dermal sclerosis was inconspicuous and there was no evidence of vasculitis. Perilesional skin gave immunofluorescence results consistent with SLE: faint but definite granular basement membrane zone staining with IgM and bright antinuclear antibody staining in the epidermis with IgG. Staining for human papilloma virus using an immunoperoxidase technique was negative.

Lupus serology. DNA binding 71 U/ml (normal 0–20); anti-nuclear antibodies positive at a titre of 1:640 (speckled pattern); complement C_3 and C_4 levels although previously reduced, were recently within normal limits.

The serum zinc level is normal.

Treatment and progress. Her general condition remains stable, with thrice weekly haemodialysis, 20 mg prednisolone daily, ranitidine and vitamins. The palms and soles are improving with fluocinonide 0·05% ointment under occulsion. The warty areas are responding to intralesional triamcinolone.

Discussion. Wong[1] recognized hyperkeratosis of palms and soles as a relatively common feature of SLE in the Chinese. When present it is often associated with other hyperkeratotic features such as follicular, hyperkeratotic papules and psoriasiform lesions. Papulo-nodular lesions resembling keratoacanthomas or hypertrophic lichen planus are seen in discoid LE.[2] We have found no reference to the combination of skin features seen in our patient. The ichthyosis alerted us to the possibility that dietary deficiency may be contributing to the pathogenesis of these unusual features.

REFERENCES

1 Wong KO. Systemic lupus erythematosus: report of 45 cases with unusual clinical and immunological features. *Br J Dermatol* 1967; **81:** 186–90.

2 Uitto J, Santa-Cruz DJ, Eisen AZ, Leone P. Verrucous lesions in patients with discoid lupus erythematosus. *Br J Dermatol* 1978; **98:** 507–20.

(16) Chronic discoid lupus erythematosus with mepacrine pigmentation bands in the nails

DRS H.J.DODD AND I.SARKANY

J.W., male, aged 40 years.

History. This patient first noted a facial eruption in 1969. He found that sunshine led to a tightness and soreness of the face. He was referred to the Royal Free Hospital in 1977 as a number of treatments had had limited success. These included topical steroid creams, sunscreens, hydroxychloroquine, local triamcinolone injection, flurandrenolone tape and sodium aurothiomalate injections.

Examination. There were large plaques of discoid lupus erthematosus over the face, nose and arms, with hypopigmentation, atrophy and scarring. During the summer of 1987, a horizontal dark banding was noted in the nails. He is normotensive and on physical examination is, otherwise, normal.

Investigations. Antinuclear antibodies were absent and the DNA binding was normal. The haemoglobin, white cell count and platelets were all normal as was hepatic and renal function.

Skin Biopsy. The epidermis was hyperkeratotic with atrophy of the stratum Malpighii and balloon degeneration of the basal layer. The dermis contained a patchy infiltrate of lymphocytes around the adnexae. Direct immunofluorescence studies on lesional skin were compatible with lupus erythematosus.

Management. The disease has been moderately well controlled with mepacrine, prednisolone, 0·05% fluocinonide cream (Metosyn®, Stuart) and sunscreens. The dose of mepacrine has been 200 mg per day since 1982, but the dose of prednisolone has been varied between 5 and 30 mg on alternate days according to the level of disease activity.

Comment. Blue-grey discolouration of the nails is a recognized complication of mepacrine treatment and was first described in patients receiving prolonged courses of the drug for malaria prophylaxis.[1] However, there is some controversy as to the exact nature of the pigment responsible.[2] Our patient has been on the same dose of mepacrine for 5 years and we are puzzled about the reason for the banding. A melanosis affecting only sun exposed areas has been described with another antimalarial, amodiaquine,[3] so it is possible that seasonal factors have influenced the pigmentation in our patient's nails. The variation in systemic steroid dosage may also have affected the pigment deposition or nail growth.

REFERENCES

1 Barr JF. Subungual pigmentation following prolonged Atabrine therapy. *US Nav Med Bull* 1944; **43:** 929.
2 Butterworth T, German JE. Discoid lupus erythematosus with peculiar pigmentation. *Arch Dermatol.* 1962; **86:** 685–6.
3 Young E. Melanosis caused by Camoquin. *Dermatologica* 1958; **116:** 389–95.

(17) Extensive linear epidermal naevi and polyostotic fibrous dysplasia

DRS M.H.A.RUSTIN, C.B.BUNKER, J.J.H.GILKES, T.W.E.ROBINSON AND PAULINE M.DOWD

University College and Middlesex School of Medicine, The Middlesex Hospital, London

S.M., male, aged 35 years.

History. This patient was born with a pigmented birthmark extending down the anterior midline and left arm. Rickets was diagnosed at the age of 2 and he was treated with vitamin D for 1 year. At the age of 8 he fractured the left humerus, radius and ulna, and 2 years later was noted to have short stature. He fractured the left femur at the age of 15 and following a left hemithyroidectomy for medullary carcinoma of the thyroid, at the age of 18, he was referred to the Royal National Orthopaedic Hospital because of musculoskeletal pains. Osteomalacia was confirmed by iliac crest biopsy and he was treated with calciferol. Despite correction of biochemical abnormalities and Looser's zones, the musculoskeletal pains continued and polyostotic fibrous dysplasia was diagnosed.

Examination. A linear verrucous epidermal naevus extends from the neck to the pubic region and down the anterior surface of the left leg. On the anterior surface of the left arm there is another epidermal naevus having both linear and whorled components, with hyperkeratosis on the palm and digits.

Investigations. Plasma calcium, phosphate, alkaline phosphatase and thyroid function tests are all normal. A skeletal survey shows gross abnormalities of the bony texture and modelling and a large defect of the left parietal bone consistent with fibrous dysplasia.

Histology. A skin biopsy shows a sharply demarcated hyperkeratotic acanthotic epidermal naevus.

Comment. This patient with polyostotic fibrous dysplasia has the hitherto undescribed association of a linear epidermal naevus and the rarely reported association of hypophosphataemic osteomalacia. Parts of the epidermal naevus have been excised, and etretinate 25 mg daily has been commenced to reduce the symptomatic hyperkeratosis. We are considering CO_2 laser therapy to the other cosmetically disfiguring areas.

(18) Pachydermoperiostosis with anaemia and splenomegaly

DRS J.K.SCHOFIELD, K.GRICE AND J.KEARNEY

Hemel Hempstead General Hospital

F.K., male, aged 35 years.

History. From the age of 20 years the patient had noticed a change in his facial features in association with swelling of the fingers, toes, knees and ankles. He also complained of persistent sweating. He had secondary infertility of 11 years duration following the birth of his first child.

Examination. There was marked coarsening of the facial features with deep furrowing of the forehead and hypertrophy of the eyelids and conjunctivae. The scalp demonstrated cutis verticis gyrata. Tripe palms and gross clubbing of fingers and toes were seen in addition to cylindrical swelling of the lower limbs. There was little axillary hair and the testes were small. The spleen was enlarged to 3 cm below the left costal margin.

Investigations. X-rays of the lower legs and forearms showed subperiosteal new bone formation resulting in marked irregular cortical thickening. Similar changes affected the tubular bones of the hands and feet. The haemoglobin concentration was 11·6 g/dl with normal white cell and platelet counts. A peripheral blood film showed a leukoerythroblastic anaemia, microcytosis and tear-drop cells. The serum ferritin was normal. An iliac crest trephine biopsy showed a moderate increase in the number of megakaryocytes with some clustering. There was a diffuse increase in reticulin. FSH and LH were raised to 18 and 24 IU/l, respectively. Testosterone was within the normal range at 16·5 nmol/l.

Comment. The patient shows the classical clinical features of primary pachydermoperiostosis. The occurrence of bone marrow failure in this condition has been reported previously,[1] albeit rarely, and it is possible that it represents part of the primary condition rather than a chance association.

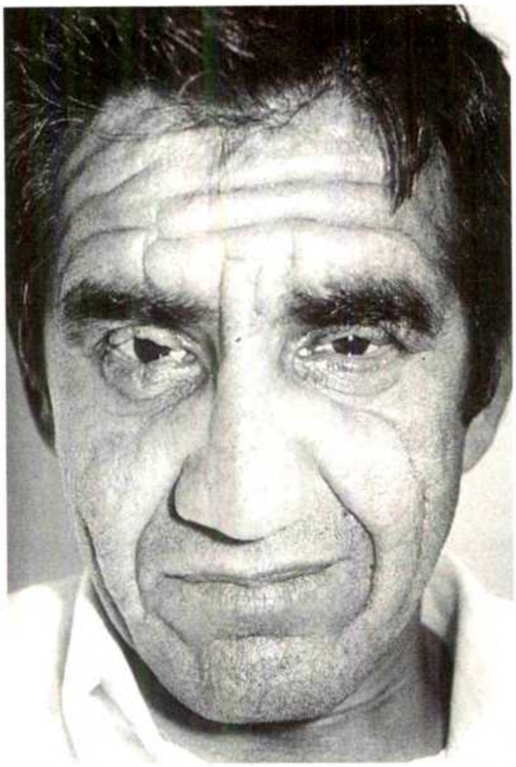

FIGURE 1. Facial appearance showing furrowing of forehead and hypertrophy of the eyelids. The patient gave written consent to the publication of an unretouched photograph.

REFERENCE

1 Metz EN, Dowell A. Bone marrow failure in hypertrophic osteoarthropathy. *Arch Intern Med* 1965; **116:** 759–64.

(19) Multiple minute digitate hyperkeratoses

DRS J.K.SCHOFIELD, K.GRICE AND N.P.SMITH*

*Watford General Hospital and *St. John's Hospital, London*

S.S., female, aged 65 years.

History. The patient presented at the age of 51 years with an 18-month history of rough skin and small filiform keratotic lesions over the anterior chest and sacrum. Over the next 14 years the lesions spread extensively over the trunk and limbs with sparing of the face. On the back the lesions became flat-topped keratotic plaques. The condition was asymptomatic. There was no significant family history and there are eight unaffected siblings. There was no history of ingestion of arsenic or other long-term medication.

Examination. The skin felt like a rough nutmeg grater. Two types of lesion were seen. There were minute filiform hyperkeratoses seen in the axillae, neck, waist, flexures of the arms and posterior aspect of the knees. Flattened hyperkeratoses of more recent onset were present on the chest, back and abdominal wall. Hair and nails were normal and the patient was in good general health.

Histology. A biopsy of the minute filiform lesions showed focal tenting of the epidermis with overlying compact orthohyperkeratosis.

Comment. This patient shows the clinical and histopathological features compatible with the condition of multiple minute digitate hyperkeratoses which was first described by Goldstein in 1967.[1] However, unlike the original case described, our patient now shows two types of lesion, namely flat keratotic papules and filiform spicules. In this respect there is a resemblance to the three cases which were described as minute aggregate keratoses by Shuttleworth *et al.*[2]

REFERENCES

1 Goldstein N. Multiple minute digitate hyperkeratoses. *Arch Dermatol* 1967; **96:** 692–3.
2 Shuttleworth D, Graham-Brown RAC, Hutchinson PE. Minute aggregate keratoses—a report of three cases. *Clin Exp Dermatol* 1985; **10:** 566–71.

(20) Scleredema of Buschke associated with multiple myeloma

DRS J.A.SALISBURY AND I.M.LEIGH

The London Hospital, Whitechapel, London

M.F., female, aged 77 years.

History. In October 1986 this patient developed stiffness and pain in her chest, hands and feet, of such severity and rapid onset over 24 h that she thought she was having a stroke. Over the next fortnight it became increasingly difficult for her to open her mouth to talk, or admit a spoon while eating.

A diagnosis of scleredema of Buschke was made. Investigation revealed a co-existing alpha heavy chain multiple myeloma. She has been treated with a course of six pulses of 750 mg cyclophosphamide intravenously at 3-weekly intervals, accompanied by prednisolone 30 mg daily for 3 days, with progressive improvement in skin mobility.

Examination. She has symmetrical woody thickening of the skin over the shoulders, neck and face. Cervical movements are limited. Her hair is sparse due to treatment with cyclophosphamide.

Skin biopsy. This showed thickening of the dermis with dense fibrosis and fenestration between collagen bundles. Stains for mucin were negative.

Investigations. The ESR was 35 mm/h. Immunoglobulins: IgA, 814 mg/dl (raised); IgG, 463 mg/dl, IgM, 13 mg/dl. Serum protein electrophoresis showed an abnormal M-band in the gamma region, composed of alpha heavy chains. The bone marrow was infiltrated with 15–20% plasma cells. Urine examination was positive for Bence-Jones protein (kappa light chains). A lateral skull X-ray showed multiple lytic lucencies in the calvarium.

Comment. Although there have been frequent reports of the association of scleredema of Buschke and benign gammopathies, there are only two other reports of an association with multiple myeloma.

REFERENCE

1 Ota A, Uitto J, Oikarninen AI. *et al.* Paraproteinaemia in patients with scleredema. *J Am Acad Dermatol* 1987; **16:** 96–107.

(21) Autoimmune progesterone dermatitis responding to tamoxifen

Drs C.J.M.Stephens, J.D.Wilkinson and F.T.Wojnarowska*

*Dowling Skin Unit, St. Thomas' Hospital, London and *Wycombe General Hospital*

C.C., female, aged 36 years.

History. The patient has suffered from a severe recurrent vesicular eczema since the age of 16 years. Menarche occurred at the age of 15, and the onset of her eruption coincided with the ingestion of an oestrogen progesterone pill, prescribed to regularize her periods.

The eczema has followed a cyclical pattern, with premenstrual flares affecting the hands, wrists and right knee, and a tendency to improve spontaneously once menstruation starts.

In addition, there was marked improvement during two pregnancies, with aggressive flares post partum.

Investigations. Histology showed a non-specific dermatitis which was negative on immunofluorescence. Intradermal tests to synthetic progesterone were negative, but intramuscular challenge produced a flare of eczema within 4 days. Oral progesterone produced a definite deterioration in the condition.

Management. The eczema has been remarkably resistant to conventional treatment with, at best, only moderate improvement with oral prednisolone 30 mg per day combined with potent topical steroids under occlusion.

Oestrogen therapy has had no effect, but oral tamoxifen 10–30 mg per day has produced complete and prolonged clearance.

Comment. Autoimmune progesterone dermatitis is frequently associated with prior exposure to synthetic progesterones, which are thought to act as a stimulus for antibodies which then cross-react with natural progesterone and perpetuate the immune response premenstrually. Oestrogen therapy is usually successful in this condition, but tamoxifen has been a useful alternative in our patient. Tamoxifen is thought to suppress ovulation, and the post-ovulatory rise in progesterone levels.

REFERENCE

1 Hart R. Autoimmune progesterone dermatitis. *Arch Dermatol* 1977; **113**: 426–30.

(22) Cicatricial pemphigoid, autoimmune neutropenia and thyrotoxicosis

Drs A.L.Davis and F.T.Wojnarowska

Wycombe General Hospital

J.L., female, aged 60 years.

History. For 18 years this patient has had idiopathic neutropenia. For 16 years she has had recurrent mouth and genital ulceration, and conjunctivitis which has resulted in scarring of the conjunctiva.

Recently, she has developed nasal fissuring, crusting and bleeding.

In 1986 she complained of weight loss and of feeling hot, and investigations confirmed that she had thyrotoxicosis.

Examination. There are multiple ulcers of the mucous membranes of the mouth with angular cheilitis. There is ulceration of the vulva, redness of the conjunctiva with some surrounding eczema and marked scarring, and fissuring, crusting and bleeding of the nose.

Investigations. Neutrophils: $0{\cdot}8 \times 10^{12}/l$. No anti-neutrophil antibodies were detected. ANF was negative. Thyroid microsomal antibodies were present at a titre of 1:1,638,400.

Pemphigus and pemphigoid antibodies were negative.

Histology. Skin, lip and buccal mucosa biopsies were negative on direct immunofluorescence.

Treatment. The neutropenia did not respond to a 9-month course of azathioprine. At a dose of 300 mg/day her mucosal disease persisted. Exacerbations of her cicatricial pemphigoid have been responsive to topical steroids. She has been treated with radioiodine and thyroxine replacement for her thyrotoxicosis.

Comment. Cicatricial pemphigoid is regarded as an autoimmune bullous disease. However, there have been no previous reports of its association with other autoimmune diseases. In this case cicatricial pemphigoid was associated with a probable autoimmune neutropenia and thyrotoxicosis.

(23) Cicatricial pemphigoid associated with auto-immune disease

DRS C.REID, F.WOJNAROWSKA AND P.A.FRITH*

*The Slade Hospital, Oxford and *The Radcliffe Infirmary, Oxford*

N.C., female, aged 61 years.

History. This woman developed vitiligo in 1952 and primary hypothyroidism in 1977 and presented in March 1986 with blistering in the mouth, bleeding gums and a history of recurrent red eyes.

Examination. Extensive vitiligo involved the face, trunk and limbs. There was variable palatal, gingival and vulval ulceration. Conjunctival erosions were present without overt scarring.

Schirmer's test was normal.

Investigations. There was staining for IgG and C_3 on skin, lip and conjunctival tissue. Indirect immunofluorescence was negative on skin and oral mucosa, and positive on conjunctiva. Gastric parietal and salivary gland antibodies were found on auto-immune screening.

Treatment. She is currently on dapsone and prednisolone for control of the cicatricial pemphigoid. Azathioprine was withdrawn when she developed cholestatic hepatotoxicity.

Comment. We present this case as an interesting combination of auto-immune related phenomena—cicatricial pemphigoid, vitiligo and primary hypothyroidism—occurring in the one patient. The association of cicatricial pemphigoid with auto-immune disease has not been reported previously.

(24) Chrysiasis

DRS VANESSA VENNING, F.WOJNAROWSKA AND P.R.MILLARD*

*Slade Hospital and *John Radcliffe Hospital, Oxford*

P.W., female, aged 78 years.

History. She presented in October 1986 with pneumonia and pleurisy and was noted, incidentally, to have slate grey discolouration of the skin, predominantly affecting the forehead and cheeks and the backs of the hands. She had been unaware of any colour change and there were no other symptoms. She had had rheumatoid arthritis for many years and for the past 8 years had received Myocrisin 50 mg/week (cumulative dose exceeding 20 g). Her other medications were prednisolone, piroxicam, temazepam and thyroxine.

Investigations. A skin biopsy showed black granular deposits of gold in the dermis, which on electron microscopy were seen to lie within the lyzosomes of macrophages. There was no increase in basal layer melanin. The presence of gold in the skin was confirmed by a laser microprobe.

Comment. Grey pigmentation confined to sun exposed skin is a recognized though rare complication of high cumulative doses of gold (chrysiasis). There may be a latent period of months or years between stopping gold treatment and the appearance of the pigmentation; furthermore the pigmentation may progress despite stopping the drug. Clinically, chrysiasis may be confused with argyria, but they can be distinguished by light and electron microscopy. Unlike silver, gold deposits are all intracellular and there is little or no increase in basal melanin.

(25) Argyria

DRS G.DOOTSON, P.WILSON AND I.SARKANY

M.J., female, aged 74 years.

History. This patient presented in 1979 with cutaneous pigmentation, most marked over sun-exposed areas. The depth of pigmentation has gradually increased, despite the absence of an obvious causative agent.

Examination. A metallic, pigmented discolouration of the entire skin surface, which is most prominent over the face and hands (Fig. 1). The nails and mucous membranes are also involved.

Histology. There is a diffuse scattering of fine black granules throughout the dermis, with a tendency to cluster around adnexal structures (Fig. 2). Electron microscopic, EDAX analysis (energy dispersive analysis of X-rays) has shown that these granules contain silver and sulphur, with a trace of selenium.

Investigations. Hepatic, renal and myocardial function is normal. Ophthalmological examination revealed silver deposits in the sub-conjuctival tissues, but no intra-ocular involvement.

Comment. We have not been able to identify the source of this lady's silver ingestion in spite of repeated attempts to do so.

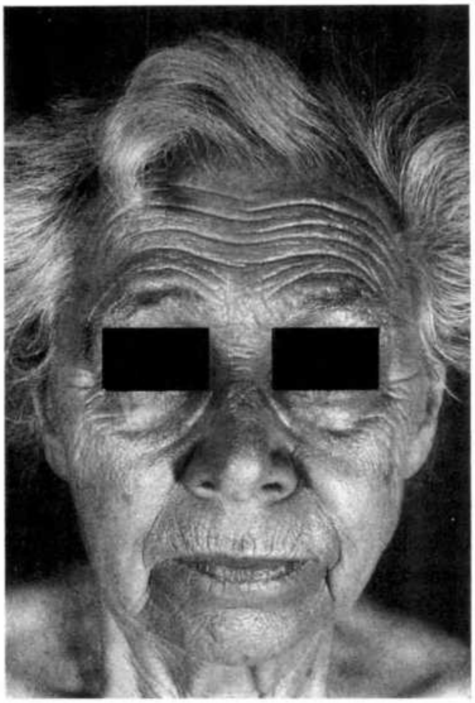

FIGURE 1. Heavy slate-blue discolouration of the facial skin.

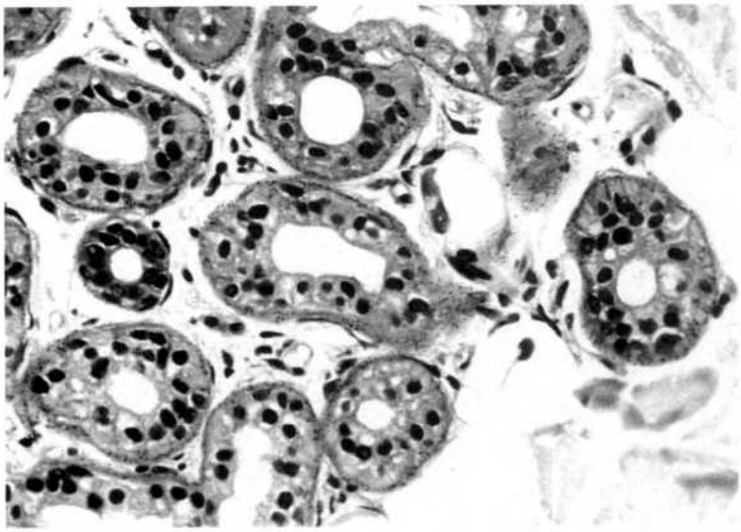

FIGURE 2. Typical grain-like metallic deposits concentrated around eccrine gland tissue.

(26) Xeroderma pigmentosum variant

DRS J.BERTH-JONES AND R.A.C.GRAHAM-BROWN

Leicester Royal Infirmary

AM., male, aged 62 years.

History. At the age of 21 years, after completion of a 5-month period of military service in Palestine, this patient developed a growth on the side of his nose. He has, subsequently, developed at least 34 further neoplastic lesions on his face and the dorsal aspects of his hands.

In his youth he developed a tan very easily after 4–6 h of exposure to minimal sunshine, and was never aware of any undue sensitivity to the sun. Since the initial lesions appeared he has avoided sun-exposure carefully.

There is no family history of skin tumours.

Examination. The skin of his face and hands has become markedly freckled and telangiectatic; numerous scars are now present.

Investigations. Testing for photosensitivity was performed at St John's Hospital in 1969. He was found to be mildly hypersensitive to UVB (300 nm), but had normal reactions at 260 nm and from 320 to 700 nm.

DNA repair synthesis induced in the patient's fibroblasts by germicidal UV irradiation is normal, confirming that he does not have an excision repair defect. Further investigation to confirm a post-replication repair defect is being undertaken.

Histology. His records at Leicester contain 37 pathology reports from suspected neoplastic lesions. Among these lesions are one malignant melanoma, eight squamous cell carcinomas, two basal cell carcinomas, three solar keratoses, and 13 keratoacanthomas.

Normal skin from the back and perilesional skin has shown connective tissue changes compatible with xeroderma pigmentosum.

Treatment. He has now undergone at least 90 surgical procedures for skin neoplasms, received radiotherapy on six occasions and innumerable treatments with cryotherapy and topical 5-fluorouracil. He is currently receiving etretinate 25 mg daily.

Comment. This patient has the clinical and histological features of xeroderma pigmentosum which began at the unusually late age of 21 years. His dermal fibroblasts show normal excision repair activity following irradiation. He, therefore, fits the diagnostic criteria for the variant form of xeroderma pigmentosum.

(27) Prolidase deficiency

DRS A.MILLIGAN AND R.GRAHAM-BROWN

Leicester Royal Infirmary

B.S., female, aged 45 years.

History. This patient presented in 1960, aged 18 years, complaining of swelling and ulceration of both legs. Multiple telangiectasia and areas of atrophie blanche were present on the legs and thighs. She was noted to have unusual facies with prominent epicanthic folds. She had had pulmonary tuberculosis at the age of 10 years.

Her ulcers have persisted in spite of therapy, including several periods of in-patient

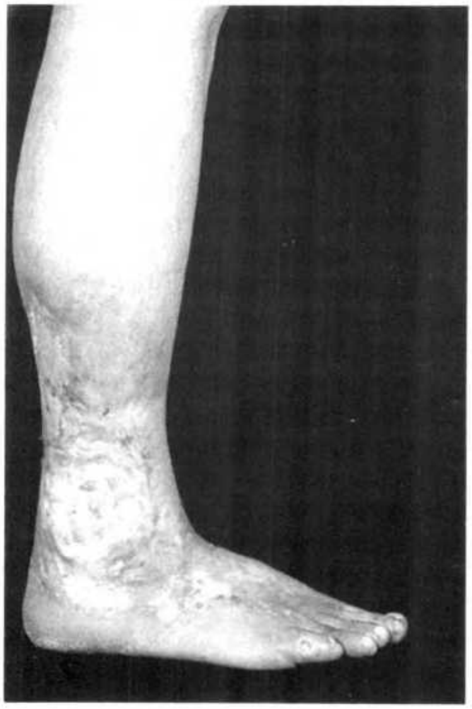

FIGURE 1. Extensive ulceration around the right ankle and dorsum of the foot.

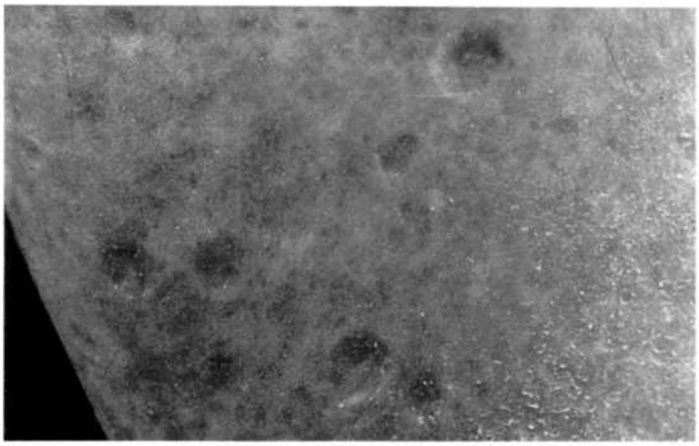

FIGURE 2. Areas of atrophic scarring on the thigh.

treatment. Since 1978 she has had recurrent episodes of 'cystitis' with only occasional isolation of bacterial pathogens.

Examination. There are bilateral leg ulcers with telangiectasia and multiple areas of atrophie blanche on her legs and thighs.

Investigations. In 1961 raised urinary levels of proline and hydroxyproline were detected. In 1968 chromosome studies were normal. Skin biopsies taken in 1977 showed thickened collagen bundles on light microscopy.

Collagen studies showed a low skin collagen content in normal and ulcerated areas. Large collagen aggregates were less stable in ulcerated areas.

Electron microscopy of a biopsy from an ulcerated area showed very marked cross banding and a 'shredded' appearance along the collagen fibres. No abnormality was seen in clinically normal skin.

In 1981 urine dipeptide levels were greatly increased and red cell prolidase levels were low. In 1984 cystoscopy and biopsy showed chronic inflammation.

Comment. Prolidase deficiency is a very rare cause of leg ulcers. It is inherited as an autosomal recessive trait. Our patient's brother has low red cell prolidase levels, but has no features of the disease. He may be a heterozygote. There are no previous reports of cystitis in prolidase deficiency.

(28) Giant congenital pigmented naevus

DRS H.J.DODD AND I.SARKANY

C.M., female, aged 26 years.

History. This patient was noted to have large pigmented areas of skin from birth. She was originally referred to the Royal Free Hospital 2 years ago for genetic counselling, regarding suspected 'Von Recklinghausen's disease'.

Examination. She was found to have a giant hairy pigmented naevus covering the lower trunk and thighs as well as smaller similar naevi scattered over the limbs (Fig. 1). There was no evidence of neurofibromatosis or of any neurological abnormality.

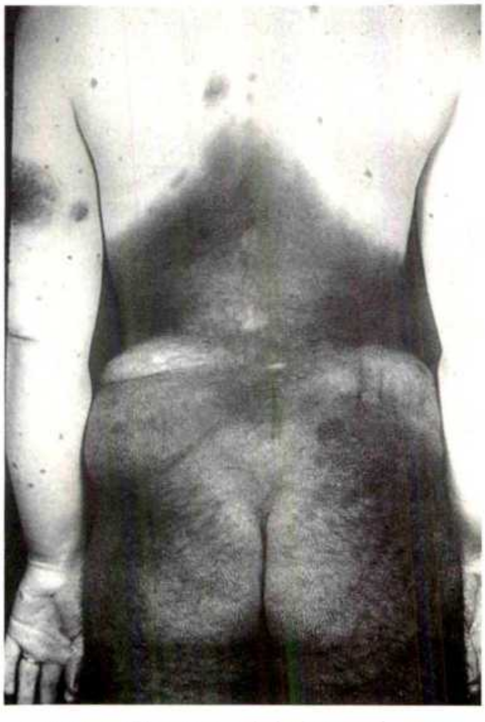

FIGURE 1. Giant congenital pigmented naevus.

Skin biopsy. This showed a melanocytic naevus extending throughout the superficial dermis. A small area of junctional activity was present, but there was no evidence of malignancy.

Management. This patient is reviewed annually with the assistance of clinical photographs. She has been advised to use sunscreens.

Comment. Although the incidence of malignant change in these naevi is considerable, our patient has accepted her disability and lives a full life. Prophylactic plastic surgical removal is not considered practical.

(29) Yellow nail syndrome

DRS G.DOOTSON AND I.SARKANY

D.J., female, aged 80 years.

History. This patient presented in 1977 with a yellow discolouration of the nails. They had also been noted to be slow growing. She has, subsequently, developed bronchiectasis and lower limb oedema. She also has a past history of atopic eczema.

Examination. All the nails are thickened, excessively curved and have a yellow discolouration (Fig. 1). Occasionally nails are lost, but they regrow. Ankle oedema and signs of bronchiectasis are present.

Investigations. A chest X-ray shows changes of bronchiectasis with some volume loss in the left lower lobe. Pulmonary function tests show some restriction of both lung volumes and gas transfer. Nail culture for fungi and yeasts was negative.

Comment. This patient demonstates the complete triad of the yellow nail syndrome, i.e. yellow nails, lymphoedema and respiratory tract involvement in the form of bronchiectasis.

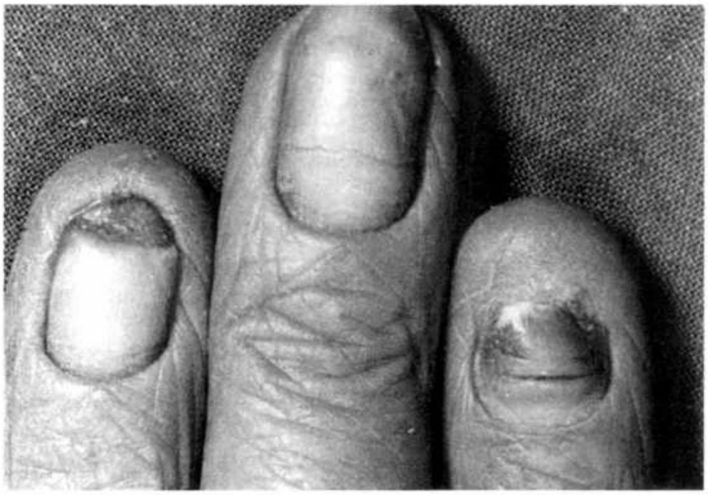

FIGURE 1. Dystrophic nails in yellow nail syndrome.

(30) Conjunctival melanoma

DRS G.DOOTSON AND I.SARKANY

N.R., male, aged 34 years.

History. This patient presented in July 1986 with psoriasis involving the scalp, beard area, palms, soles, nails and sites of previous trauma. He was coincidentally noted to have a raised, pigmented lesion laterally on the left conjuctiva (Fig. 1).

Histology. The appearances were those of a conjunctival melanoma. Plump spindle-shaped cells, which were heavily pigmented penetrated into the overlying epithelium. The maximum thickness was 0·8 mm. Evidence of primary acquired melanosis was present in the tissue surrounding the melanoma.

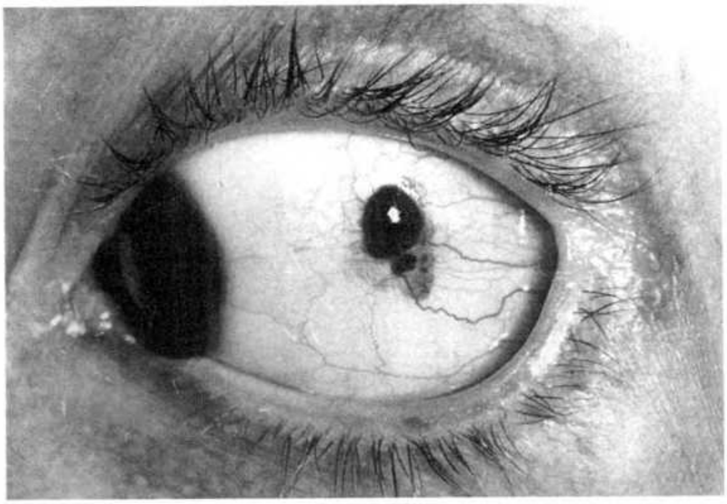

FIGURE 1. Raised, heavily pigmented conjunctival melanoma.

Investigations. There has hitherto been no evidence of dissemination on chest X-ray, and hepatic and bone scans.

Treatment. The lesion was completely excised by our eye surgeon.

Comment. Primary acquired melanosis has variously been reported to progress to melanoma in 17–46% of cases. Tumour thickness, although showing some correlation with prognosis, is not as predictive of outcome as with cutaneous melanoma. Metastases occurring from thinner lesions are thought to occur via the relatively superfical lymphatics in the conjunctiva. Although an accurate figure for incidence of this rare condition is not available, a clinico-pathological study of 131 cases of conjuctival melanoma has provided information on the proportion of cases of primary acquired melanosis progressing to melanoma and on the features which help to predict metastatic spread.[1]

REFERENCE

1 Folberg R, McLean IW, Zimmerman LE. Conjunctival melanosis and melanoma. *Ophthalmology* 1984; **91:** 6; 673–8.

(31) Classical Kaposi's sarcoma and squamous cell carcinoma of the leg

DRS G.DOOTSON AND I.SARKANY

S.C., female, aged 85 years.

History. This lady presented 20 years ago with nodular lesions of Kaposi's sarcoma over both feet. The lesions have subsequently merged to form plaques over the feet and ankles, with new nodules appearing up to mid thigh. Lymphoedema of both legs has developed. In 1984 an eroded area on the right shin was confirmed to be squamous cell carcinoma.

Examination. There are numerous purple nodular and plaque-like lesions of Kaposi's sarcoma over both feet and legs.

Histology. The appearances are typical of Kaposi's sarcoma, with spindle cells, slit-like vascular spaces and extravasation of red blood cells. Immunoperoxidase staining for vimentin and factor eight related antigen is positive.

Treatment. The patient has received two courses of radiotherapy to plaque-like lesions on the ankles. 2250 CGy was given in five fractions on each occasion, from a cobalt 60 unit. The squamous cell carcinoma was treated with radiotherapy (4850 CGy at 120 kV), but the site subsequently required grafting due to ulceration. Support hose has provided some relief from the lymphoedema.

Comment. This case demonstrates the indolent progression of classical Kaposi's sarcoma, with the characteristic development of lymphoedema. An association with lymphomas and other malignancies is well recognized, although previous reports of squamous cell carcinoma are exceedingly rare. In this patient, radiotherapy to the Kaposi lesions followed the development of the squamous cell carcinoma and radiation could not, therefore, be incriminated in the aetiology.

(32) Tuberous sclerosis

DRS H.J.DODD AND I.SARKANY

H.C., female, aged 41 years.

History. This patient developed epilepsy during her first pregnancy in 1963, but for the last few years her attacks have been nocturnal. Many years ago one toe was amputated after a skin lesion became septic. She was referred to the Dermatology Department in 1984 with an enlarging compound melanocytic naevus and the diagnosis of tuberous sclerosis was made.

Family history. Her son also has epilepsy and is said to show some cutaneous features of the condition. He has not been examined.

Examination. She was noted to have angiofibromata over the nose as well as multiple periungual fibromata (Fig. 1). A shagreen patch was present in the lumbar area and there was a leaf-shaped area of hypopigmentation on the right arm. Neurological examination, including fundoscopy, revealed no abnormality.

Investigations. A skull X-ray was normal, but an electroencephalogram showed a well marked temporal abnormality indicative of a potentially epileptogenic organic disturbance.

Histology of the naevus excised from the abdomen showed a benign compound naevus with prominent junctional activity.

Comment. This patient shows all the pathognomonic skin lesions of tuberous sclerosis. Fortunately, late onset epilepsy has been the only non-cutaneous manifestation of the disease in this case and the convulsions have been easily controlled by phenytoin 100 mg daily.

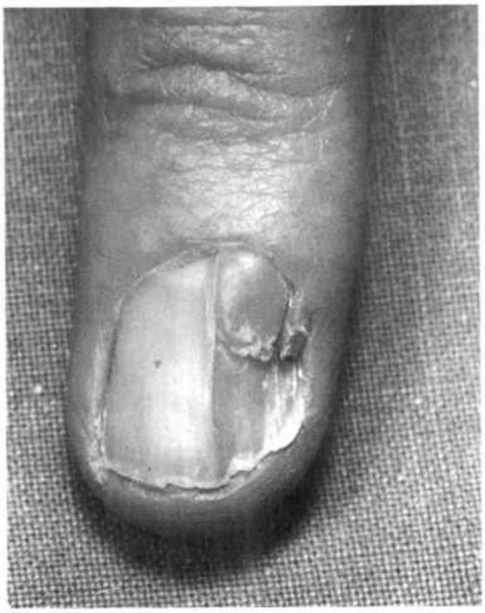

FIGURE 1. Koenen's tumours arising from the nail fold.

(33) Exfoliative cheilitis

DRS H.J.DODD AND I.SARKANY

P.M., male, aged 26 years.

History. This patient was first seen in July 1984 with a 7-year history of cracked hard skin on the lips with associated crusting. The lips bled at times and discomfort had interfered with eating. He also suffered from allergic rhinitis and mild atopic eczema. There was a strong family history of atopy.

Examination. The lips were covered with thick, yellow, fissured hyperkeratotic debris.

Investigations. Patch-testing was negative. Culture of a lip swab gave a profuse growth of *Staphylococcus aureus*.

Treatment. In-patient treatment with oral erythromycin and 0·1% triamcinolone ointment with 3% tetracycline (Aureocort®, Lederle) produced a great improvement.

Comment. Exfoliative cheilitis is an uncommon condition which is resistant to treatment. Affected subjects are often atopic and emotional disturbances are frequently found.

(34) Primary facial lymphoedema with xanthomas

F.M.Tatnall and I.Sarkany*

*Department of Dermatology, The London Hospital Whitechapel and *Department of Dermatology, The Royal Free Hospital, London*

J.C., female, aged 48 years.

History. In 1981 the patient developed swelling of the face, ears and scalp which has persisted, although it fluctuates from day to day.

Examination. The patient has bilateral periorbital oedema, brawny induration of the cheeks and swelling of both ears. There are xanthomas on the ears and in the preauricular region.

Investigations. The following were normal or negative: urine analysis for blood, 24 h urine excretion of protein, full blood count, urea and electrolytes, creatinine clearance, serum C_1 esterase inhibitor, serum thyroxine and serum protein electrophoresis. A fasting lipid profile showed the following results: cholesterol 10·8 mmol/l (normal 3·0–6·5 mmol/l), triglyceride 1·6 mmol/l (normal 0·2–1·6 mmol/l). Lipoprotein electrophoresis showed an increased β band, a pattern consistent with a Fredrickson type IIa hyperlipidaemia. Lipid studies performed on the patient's family were normal.

Treatment. The patient has been treated with bezafibrate 200 mg t.d.s. and the xanthomas have partially resolved.

Comment. This patient has lymphoedema of the head which, in the absence of underlying disorders, we believe to be primary. Xanthomas are a rare complication of primary lymphoedema, but reported cases have been normolipaemic. Our patient differs in having a polygenic hypercholesterolaemia, a condition not normally associated with such extensive xanthomas. Chronic lymphoedema may have predisposed to the deposition of lipid within the skin, giving rise to this unusual pattern of xanthomas.

(35) Lichen aureus

Drs C.Buckley and I.Sarkany

V.D., female, aged 7 years.

History. This patient has had an asymptomatic brownish plaque on the medial aspect of her right ankle for approximately 2 years.

Investigations

Histology. Sections showed hyperkeratosis, expansion of the granular layer and a mild lymphohistiocytic infiltrate in the superficial dermis. Iron deposition within histiocytes was marked.

Comment. Although lichen aureus falls into the group of pigmented purpuric eruptions, there are good clinical and histological reasons for accepting it as a separate clinical and histological entity with a good prognosis.

REFERENCE

1 Price ML, Wilson Jones E, Calnan CD, MacDonald DM. Lichen aureus: A localized persistent form of pigmented purpuric dermatitis. *Br J Dermatol* 1985; **112**: 307–14.

(36) Delayed pressure urticaria, cold urticaria and chronic urticaria

FRANCES LAWLOR, A.KOBZA BLACK AND M.W.GREAVES

Institute of Dermatology, St Thomas's Hospital, London

S.F., female, aged 54 years.

History. During the summer of 1986 she noticed itchy red lesions on both legs which became more frequent over the next few months and more localized to the posterior aspects of thighs, knees and extensor aspects of forearms. Later, swelling of hands and extensor aspects of knees lasting several hours occurred following housework, gardening and shopping. Tingling is present prior to the appearance of lesions which became itchy and sometimes painful. Swellings occur daily and if present close to a joint, it may ache.

During the same period a separate transient itchy eruption was present twice weekly. Occasional transient lip swelling occurred.

For 4 years she developed 'nettle rash' after swimming in a cold sea or swimming pool with no associated systemic features.

Anaphylaxis followed a bee sting in 1987. She developed asthma during her second pregnancy in 1957 and has migraine.

Examination. Tender erythematous swellings are seen after sustained pressure on localized areas of skin.

Investigations. Using a calibrated dermographometer, pressures of $9{\cdot}75 \times 10^5$ pascals were applied to the back for 5, 15, 33, 70 and 100 s. These were positive with palpable swelling at 6 h. Immersion of the forearm in water kept at 10°C for 10 min produced wealing on rewarming. ESR and WCC have been raised on one occasion. Other investigations, including those for other forms of urticaria, were negative.

A skin biopsy showed moderate numbers of eosinophils and some neutrophils both around blood vessels and between collagen bundles. The amount of infiltrate is considerably heavier than usually found in chronic urticaria.

Treatment. Antihistamines and prednisolone taken in a dose of 40 mg daily for 1 month had no effect on her delayed pressure urticaria (DPU). Treatment with colchicine 0·5 mg b.d. has suppressed her DPU. Her cold urticaria and chronic urticaria are not sufficiently symptomatic to need treatment at present.

Comment. DPU is rare and is associated in 66% of cases with chronic urticaria. Our patient illustrates the clinical picture, histopathology and treatment response in DPU. The concurrent association of two or more physical urticarias is quite frequent which might suggest an underlying circulating factor. However, we are not aware that the co-existence of DPU and cold urticaria in the same patient has been reported before. Colchicine is an effective treatment in DPU, possibly because of its effects on neutrophil and eosinophil chemotaxis.

REFERENCES

1 Dover JS, Kobza Black A, Milford Ward A, Greaves MW. Delayed pressure urticaria, clinical features, laboratory investigations and response to therapy. *Arch Dermatol* (In press).

2 Sonin L, Grammer LC, Patterson R. The occurence of multiple physical allergies in the same patient. Report of three cases. *J Allergy Clin Immunol* 1985; 75: 705–8.

(37) Muckle Wells syndrome in a mother and children

DRS S.E.KELLY AND R.W.EMMERSON

The Royal Berkshire Hospital, Reading

S.G., female, aged 29 years; M.G., male, aged 7 years; S.G., female, aged 11 months.

History and examination. All three affected family members suffered from an identical widespread erythematous urticated maculopapular eruption. Although migratory, individual lesions persist for up to 8 h. Noted since early infancy, the rash is present on most days. The mother and son also suffer from intermittent transient limb and joint aches, but without evidence of arthritis. The mother has itchy, painful eyes with clinically a mild blepharitis. Poor hearing has been evident in both mother and son since early childhood.

Audiometry reveals a 'ski-slope' audiogram, in which hearing falls off steeply from low to high tones. The hearing loss is thought to be mostly cochlear with symmetrical 50 db loss.

Investigations. The ESR is variably raised at 30–70 mm/h. There is mild neutrophilia (WCC 13·0–17·0 × 19^9/l). ANF was weakly positive at a titre of 1 : 10 with borderline DNA binding (21 U/ml). The following were normal or negative: Serum biochemistry, immunoglobulins, cryoglobulins, complement studies, X-rays of chest, hands and feet, virus and syphilis serology. A rectal biopsy was negative for amyloid in the mother, but a renal biopsy has not been performed. Skin biopsies showed mild dermal oedema with a scattered perivascular and periadnexal infiltrate of chronic inflammatory cells. Direct immunofluorescence was negative.

Comment. This family, we believe, has Muckle Wells syndrome, a disease complex consisting of amyloidosis, deafness, urticarial rashes and migratory limb and joint pains.[1] It is thought to be inherited in an autosomal dominant fashion and this family's pattern of inheritance would support this—there is also one daughter who is not affected. Systemic amyloidosis seems to occur in about 25% of reported cases with renal failure supervening.[2] Onset is usually in late childhood, but there is a report of a family in whom the rash was noted at birth.[3]

REFERENCES

1 Muckle TJ, Wells M. Urticaria, deafness, and amyloidosis: a new heredo-familial syndrome. *Quart J Med* 1962; **31**: 235–48.

2 Black JT. Amyloidosis, deafness, urticaria, and limb pains: a hereditary syndrome. *Ann Int Med* 1969; **70**: 989–94.

3 Champion RH. Urticaria, amyloidosis and deafness. Muckle Wells syndrome. *Br J Dermatol* 1975; **93** (Suppl. 11): 46–8

(38) Systemic mastocytosis with bone pain

DRS C.B.ARCHER AND M.W.GREAVES

St John's Hospital for Diseases of the Skin, London

J.H., male, aged 49 years.

History. This man presented with a generalized maculopapular eruption in 1973, accompanied by pruritus and wealing of the lesions, either spontaneously or following minimal trauma. He has not experienced flushing, wheezing or gastrointestinal symptoms. For the past 4 years he has been troubled by low back pain, radiating to both buttocks and the anterior thighs, and exacerbated by slight movement and sneezing.

Examination. He has a widespread reddish-brown eruption, characteristic of urticaria pigmentosa, with a positive Darier's sign. There is some tenderness over the lumbar spine with no restriction of spinal movements, and straight leg raising is normal. He has no peripheral neurological abnormalities and no hepatosplenomegaly.

Investigations. A bone scan showed generalized high uptake with no focal abnormalities and a skeletal survey revealed diffuse bony sclerosis with endosteal new bone deposition and associated lucencies in the spine, long bones, pelvis and skull. There appeared to be minor posterior disc space narrowing of L4–5, but a myelogram showed no evidence of disc protrusion or nerve impingement. A liver and spleen scan was normal.

Blood tests revealed a haemoglobin of 17 g/dl with normal red cell mass and a plasma volume of 33 ml/kg (normal range 40–50 ml/kg). The plasma alkaline phosphatase level was raised at 17 KAU/dl (normal range 2–11 KAU/dl), with normal calcium and phosphate levels.

Histology. A bone marrow (trephine) biopsy showed diffuse mast cell infiltration with reactive bone changes and some fibrosis; other cell lines were normal.

Treatment. The itching and wealing improved considerably following treatment with terfenadine 120 mg b.d. plus cimetidine 400 mg b.d.[1] Sodium cromoglycate, ketotifen, topical steroids and PUVA were ineffective. The back pain did not repond to simple analgesics, physiotherapy, radiotherapy or intravenous mithramycin.[2] However, pulse therapy with intravenous methyl prednisolone 1 g daily for 5 days resulted in considerable improvement of the back pain in that his backache, when recumbent, cleared. However, the bone scan appearance did not change after treatment and the back pain recurred within 6 weeks of therapy.

Comment. Intravenous methyl prednisolone, used in the treatment of myeloproliferative disorders, was used in this patient since mast cells are known to be sensitive to high doses of corticosteroids.

REFERENCES

1 Frieri M, Alling DW, Metcalfe DD. Comparison of the therapeutic efficacy of cromolyn sodium with that of combined chlorpheniramine and cimetidine in systemic mastocytosis. *Am J Med* 1985; **78:** 9–14.

2 Conrad ME, Carpenter JT, Todd JN, Murad TM. Mithramycin in the treatment of systemic mastocytosis. *An Int Med* 1975; **83:** 659–60.

(39) Congenital erythropoietic porphyria (Günther's disease)

DRS C.B.ARCHER, V.S.NEILD* AND J.L.M.HAWK

*St John's Hospital for Diseases of the Skin, London and *Buckland Hospital, Dover*

A.B., male, aged 9 years.

History. This boy first presented in June 1983, at the age of 4 years, with blistering of the fingers and wrists. He has since developed blisters and erosions on the face, worse during the summer months, and excessive facial hair. His history is otherwise unremarkable and there is no family history of photosensitivity.

Examination. He has scarring of the dorsa of the hands and face, with blistering and erosions during the summer (Fig. 1). He also has facial hypertrichosis. At presentation he had a palpable spleen, 2 cm below the costal margin, and his urine was noted to be a pinkish colour.

Investigations. He was anaemic, having a haemoglobin of 10·9 g/dl, with nucleated red blood cells in the peripheral blood and mild neutropenia. Liver function tests were normal. The diagnosis of congenital erythropoietic porphyria was confirmed by the findings of elevated

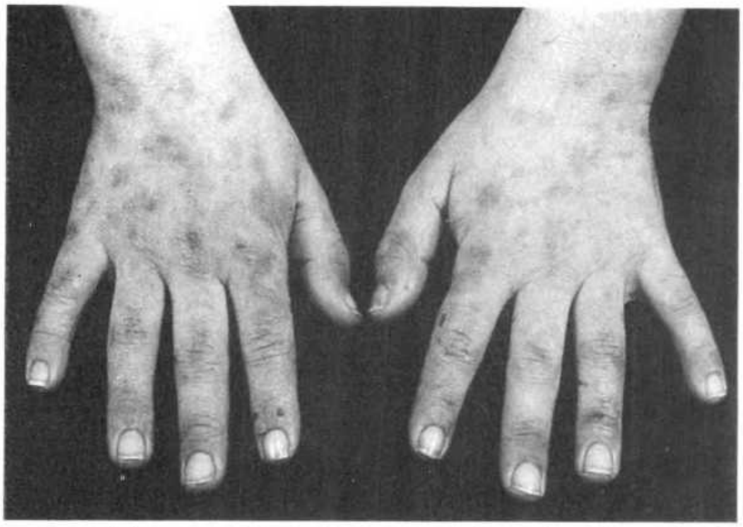

FIGURE 1. Congenital erythropoietic porphyria with erosions and scarring of the dorsa of the hands.

urinary uroporphyrin (889 nmol/24 h) and coproporphyrin (757 nmol/24 h), raised faecal coproporphyrin (957 nmol/g dry weight) and protoporphyrin (155 nmol/g dry weight) and increased protoporphyrin in blood (2849 nmol/l).

Treatment. To minimize the effects of sunlight he uses high protection factor sunscreens with efficacy against UVA and moved from a school with large windows to an older, darker school. He has been taking beta-carotene since June 1985, last year increasing the dose to 150 mg daily from April to October. Despite these measures he continues to be troubled by sun-induced blistering and erosions. The mild anaemia has not required specific therapy.

Comment. Congenital erythropoietic porphyria is a rare disease in which treatment is unsatisfactory. Haem arginate injections have been proposed as a treatment for acute hepatic porphyrias[1] and could be considered as a therapeutic option for this patient in the future if he continues to have problems.

ACKNOWLEDGMENT

We are grateful to Dr S. Williamson of Buckland Hospital for allowing us to present this patient.

REFERENCE

1 Mustajoki P, Tenhunen R, Tokola O, Gothoni G. Haem arginate in the treatment of acute hepatic porphyrias. *Br Med J* 1986; **293:** 538–9.

(40) Erythropoietic protoporphyria with hepatic involvement

DRS J.N.W.N.BARKER, R.CERIO, D.FRANCE* AND D.M.MACDONALD

*Guy's Hospital, London and *Crawley General Hospital*

L.W., female, aged 30 years.

History. This patient initially presented in the summer of 1958, aged 18 months, with intermittent redness and swelling of the face and hands. These symptoms have persisted

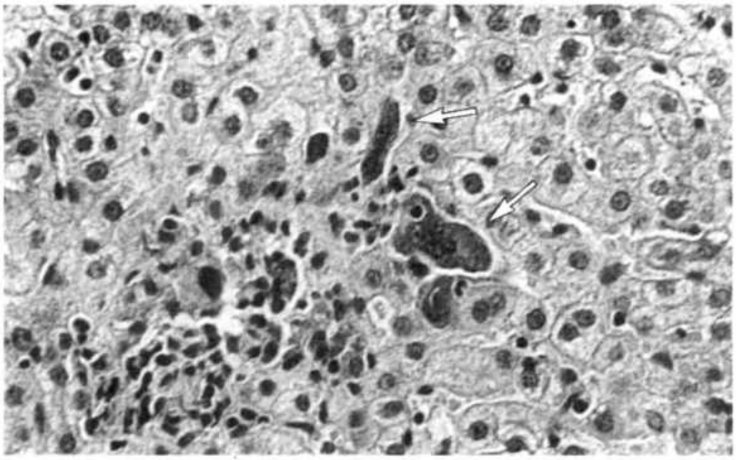

FIGURE 1. Photomicograph of liver showing deposition of protoporphyrin in hepatocytes (arrows) (original × 200).

following exposure to sunlight, with the development of atrophic waxy scarring over sun exposed areas. There was no relevant family history. Erythropoietic protoporphyria (EPP) was diagnosed in 1973 (Professor I.Magnus, St. John's Hospital). Since 1973, benefit has been obtained from a combination of sunscreens and systemic beta carotene and canthaxanthin. There have been no symptoms suggestive of hepatic disease.

Examination. There are depressed scars over the nose and cheeks, with furrowing around her lips. Waxy thickening of the skin is evident over the dorsa of her hands. In the summer months, orange discolouration of the skin is present secondary to therapy with beta carotene.

Investigations. Red cell protoporphyrin was markedly elevated at 776 μg/100 ml (normal range: $<4{\cdot}5$ μg/100 ml). Liver function tests were all normal apart from a raised bilirubin level of 35 μmol/l (normal range: <17 μmol/l). A hepatic ultrasound scan performed in 1985 revealed a diffusely abnormal liver.

Histology. A liver biopsy performed in 1978 was normal apart from minimal pigment deposition. Histopathological examination of a repeat biopsy in 1985 demonstrated deposition of protoporphyrin within the hepatocytes (Fig. 1), cholestasis, mild periportal fibrosis and lymphocyte infiltration. Electron microscopy revealed the deposition of protoporphyrin within the mitochondria of the hepatocytes.

Comment. The hepatic histopathological and ultrastructural features observed in this case are characteristic of EPP.[1] Hepatic fibrosis can frequently be present in the absence of abnormal liver function tests[2] and may herald the onset of cirrhosis. At present, there is no satisfactory therapy for severe liver disease associated with EPP.

REFERENCES

1 MacDonald DM, Germain D, Perrot H. The histopathology and ultrastructure of liver disease in erythropoietic protoporphyria. *Br J Dermatol* 1981; **104:** 7–17.

2 Cripps, DJ, Goldfarb SS. Erythropoietic protoporphyria: hepatic cirrhosis. *Br J Dermatol* 1978; **98:** 349–54.

(41) Erythropoietic protoporphyria

DRS H.J.DODD AND I.SARKANY

M.D., male, aged 29 years.

History. He has noted burning and a sense of irritation in sunshine since childhood. This interferes with his work as a home help. He takes no medication and drinks only small quantities of alcohol.

Examination. He has many ephelides and there are lesions on the dorsa of his hands resembling plane warts. The skin at these sites looks prematurely aged. There are no signs of liver disease and he has no retinal changes.

Investigations. Liver function tests are normal. Porphyrin studies show an erythrocyte protoporphyrin level of 17,000 nmol/l of packed cells (normal 0–800); faecal protoporphyrin excretion is raised at 244 nmol/g dry stool (normal 0–200). Faecal coproporphyrin is barely detectable and there is no excess of porphyrins in the urine.

Skin biopsy. There is mild acanthosis of the epidermis. In the upper dermis there are dense amorphous homogeneous hyaline areas which are PAS positive. The appearances are characteristic of porphyria.

Management. He has been treated with six capsules of beta-carotene and canthaxanthin daily during the summer months as well as with sunscreens. This has improved his tolerance of strong sunlight.

Comment. In erythropoietic protoporphyria the objective signs may be so subtle that the condition escapes diagnosis. In contrast, the symptoms described by patients may sound so inconsistently severe that a neurosis is suspected. Early gallstones and hepatic abnormalities have been described in patients with erythropoietic protoporphyria,[1,2] but our patient has no symptoms or abnormality of liver function.

Beta-carotene and canthaxanthin has proved most effective in reducing our patient's symptoms. Some patients on this treatment have developed retinal deposits of canthaxanthin, but fundoscopy in our patient is normal.

REFERENCES

1 Cripps, DJ, Scheuer PJ. Hepatobiliary changes in erythropoietic protoporphyria. *Arch Pathol* 1965; **80:** 500–8.

2 Bloomer JR, Phillips MJ, Davidson DL, Klatskin G. Hepatic disease in erythropoietic protoporphyria. *Am J Med* 1975; **58:** 869–82.

(42) Segmental neurofibromatosis with generalized café au lait spots

DRS C.B.ARCHER, M.GLOVER* AND D.J.ATHERTON*

*St John's Hospital for Diseases of the Skin and *Hospital for Sick Children, Great Ormond Street, London*

H.C., female, aged 11 years.

History. This girl first noticed increasing pigmentation and 'lumpiness' on the left side of her abdomen in 1984. These features became more extensive during the following year, being accompanied by diffuse soft tissue swelling in the same area. She was otherwise well and there is no family history of neurofibromatosis. The lesions have remained unchanged in size during the past 3 years, but she has recently complained of tingling in the area of pigmentation.

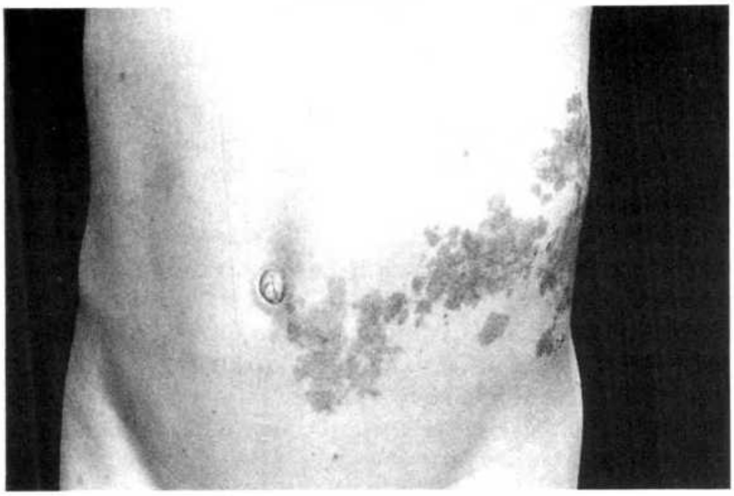

FIGURE 1. Segmental neurofibromatosis with coalescing café au lait macules overlying subcutaneous nodules.

Examination. There are numerous grouped, coalescing café au lait macules extending from the back around the left side of the abdomen to the umbilical region, in the T10–11 distribution, with a number of nodules and diffuse soft tissue swelling in the same region (Fig. 1). In addition, she has between 10 and 20 café au lait spots on the trunk, outside the segmental distribution of the nodular lesion. There is no axillary freckling and general examination is otherwise normal.

Investigations. Audiological examination and a CT scan were normal and ophthalmological examination showed no evidence of Lisch nodules. There was no macrocephaly or hypertelorism.

Histology. Biopsy of a nodule revealed an encapsulated subcutaneous tumour, comprising lobules of spindle cells and fibrous tissue surrounded by a wavy, lamellar capsule. The tumour contained areas of mucin deposition and nerve-like structures.

Comment. This patient has segmental neurofibromatosis, a disorder which may comprise a number of subsets. Despite the generalized distribution of the café au lait spots, there is, at present, no evidence of systemic neurofibromatosis.

REFERENCE

1 Roth RR, Martines R, James WD. Segmental neurofibromatosis. *Arch Dermatol* 1987; **123:** 917–20.

(43) Pustular panniculitis in rheumatoid arthritis

DRS J.NEWTON AND F.T.WOJNAROWSKA

Wycombe General Hospital

B.H., female, aged 55 years.

History. This lady has had rheumatoid arthritis since 1983. In April 1987 she developed painful red swellings on the lower legs. These later ulcerated and discharged pus. She has since developed lesions on the buttocks. In the past she has been treated with gold, penicillamine and anti-malarials. When she developed the eruption she was taking only non-steroidal anti-inflammatory tablets and having regular injections of a depot methylprednisolone preparation.

Examination. There were multiple red subcutaneous nodules, most of which were suppurating. Some lesions were ulcerated and others had healed with scarring.

Investigations. Rheumatoid factor was positive at a titre of 1:40. Anti-nuclear factor was negative. Amylase was normal. No organisms have been cultured from repeated swabs.

Histology. A skin biopsy showed fat necrosis in the subcutaneous tissue; within this area there was a prominent collection of neutrophilic dust. The surrounding tissues showed fibrosis. There was a light chronic inflammatory infiltrate around some of the smaller blood vessels. Stains for organisms, including acid fast bacilli, were negative.

Treatment. She has partially responded to treatment with dapsone and minocycline.

Comment. Although panniculitis has been described in connective tissue diseases, it is a most unusual finding in rheumatoid arthritis.

REFERENCE

1 Winkelmann RK. Panniculitis in connective tissue disease. *Arch Dermatol* 1983, **119**: 336–44

(44) Acanthosis nigricans in an hirsute woman

DRS J.H.BARTH, D.J.EVANS* AND F.WOJNAROWSKA

*Departments of Dermatology and *Endocrinology, John Radcliffe Hospital, Oxford*

S.D., aged 18 years.

History. She presented with a history of obesity in childhood which had worsened over the past 5–6 years. At the age of 12 she became aware of increased facial and body hair and the development of thickened, rough and discoloured skin at the back of her neck and axillae. She had menstruated only once at 13½ years. There was no relevant family history.

Examination. She was grossly obese (body mass index 35·0) with truncal obesity, striae and a 'buffalo hump'. There was no lipodystrophy. Her breasts and external genitalia were poorly developed, but there was normal axillary and pubic hair. She had mild acne vulgaris on the face and back, generalized severe hirsuties, generalized keratosis pilaris and acanthosis nigricans affecting the axillae, groins, neck and elbow tips. Her blood pressure was 100/70 mm Hg.

Investigations. Testosterone was 5·2 nmol/l (normal 1·0–2·1), dehydroepiandosterone 6·1 μmol/l (normal 1·7–8·7), sex hormone binding globulin 7·6 nmol DHT bound/l, she had a repeatedly raised LH:FSH ratio, Synacthen stimulated 17-OH progesterone 7·9 nmol/l (normal 0·9–18·0), prolactin 160 U/l (normal < 600), cortisol at 0900 h was 265 nmol/l (normal < 690) and at 2400 h 54 nmol/l, and excretion of urinary 17-oxogenic steroids was 35 μmol/24 h (normal 21–60).

Metabolic data. An oral glucose tolerance test demonstrated impaired glucose tolerance. The fasting insulin was markedly raised at 65 μU/l. Glucose disposal was diminished at 72 mg/m^2 surface area/min indicating severe insulin resistance. An age- and weight-matched control (aged 20 years, body mass index 35·0) had a fasting plasma glucose of 5·5 mmol/l, a fasting insulin of 24 μU/l and a glucose dispersal rate of 213 mg/m^2/min.

Ultrasound. Multiple 3–4 mm cysts were seen in both ovaries.

Comment. Acanthosis nigricans and insulin resistance are recognized to be features associated with hyperandrogenism and may occur in more than 12% of women presenting with hirsuties.[1] The acanthosis nigricans was so severe in this case that it was the presenting complaint. It has shown no tendency to regress during 2 years of anti-androgen therapy.

REFERENCE

1 Barth JH, Ng LL, Wojnarowska F, Dawber RPR. Acanthosis nigricans, insulin resistance and cutaneous virilism. *Br J Dermatol* 1988; **118:** 613–20.

(45) Acanthosis nigricans with insulin resistance and hyperandrogenism

DRS D.C.BUCK, F.M.TATNALL AND I.SARKANY

S.R., female, aged 48 years.

History. A Caucasian nurse presented in 1983 with acanthosis nigricans, hirsutism, obesity, oligomenorrhoea, hypertension, primary infertility and unilateral breast hypoplasia. Menarche was at 11 years; at 14 years the left breast stopped developing and she became hirsute. Hyperpigmentation developed in both axillae and over the left breast. At 24 years her periods became irregular and she became obese. Hypertension was diagnosed at 34 years.

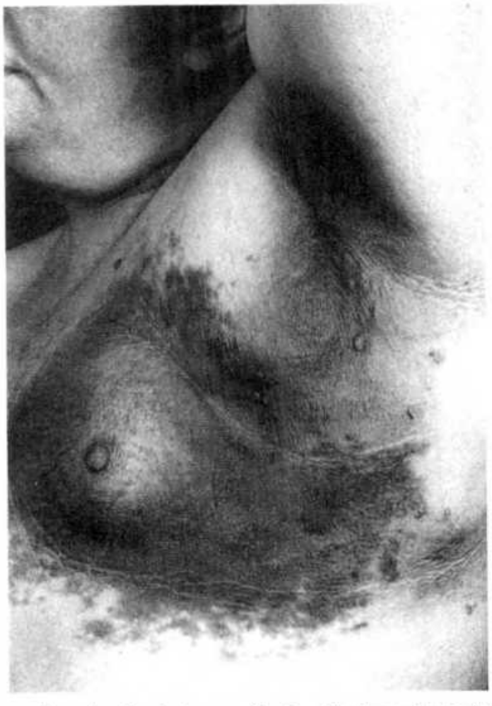

FIGURE 1. Acanthosis nigricans affecting the hypoplastic left breast.

Examination. She is obese and hirsute, and has acanthosis nigricans affecting the hypoplastic left breast (Fig. 1), axillae, antecubital fossae and neck. Her blood pressure is 160/85 mm Hg.

Investigations. Testosterone was 5 nmol/l (normal 0·7–3·1 nmol/l), 24 h urinary 17-oxosteroids were 10·2 µmol/24 h (normal 16–52 µmol/24 h). Cortisol and thyroxine levels were normal. A skull X-ray was normal. Ultrasound examination of the pelvis showed symmetrically enlarged and polycystic ovaries. Following an oral dose of 50 g glucose, insulin levels were all above 250 µU/l (normally not exceeding 47 µU/l), glucose tolerance was normal and growth hormone suppressed normally. During an insulin tolerance test 0·2 U/kg insulin (normal < 0·15 U/kg) was required to produce hypoglycaemia (glucose 1·7 mmol/l). Antibodies to insulin, antithyroid antibodies and antinuclear factor were not detected. Skin biopsies from the left axilla and left breast showed changes consistent with acanthosis nigricans.

Treatment and progress. Cyproterone acetate 100 mg daily was started from days 5 to 14 of the menstrual cycle and ethinyl oestradiol, initially 50 µg, then 30 µg daily from days 5 to 25. This improved the hirsutism and acanthosis nigricans, and aided weight reduction. Blood pressure is controlled with enalapril 10 mg twice daily, frusemide 40 mg daily and amiloride 5 mg daily. She remains well and normoglycaemic.

Comment. Acanthosis nigricans is associated with a variety of endocrine and metabolic disorders,[1] including conditions characterized by insulin resistance. Insulin resistance may be due to reduced numbers of insulin receptors expressed on cells (type A), circulating antibodies which bind to insulin receptors (type B) or to a post receptor defect. Tatnall *et al.*[2] reported the present case and one other, both with acanthosis nigricans, polycystic ovary syndrome and type A insulin resistance. Whether the increase in insulin or in androgens is the primary event in this syndrome is uncertain. Both factors may be involved in the development of the acanthosis nigricans. The involvement here of only the hypoplastic breast is of interest. Breast hypoplasia is thought to be due to end-organ resistance to circulating hormones, raising the possibility of a more generalized end-organ resistance in this patient.

REFERENCES

1 Brown J, Winkelmann RK. Acanthosis nigricans: a study of 90 cases *Medicine (Baltimore)* 1968; **47**: 33–51.

2 Tatnall FM, Graham-Brown RAC, Dandona P, Sarkany I. The syndrome of acanthosis nigricans, hyperandrogenism and insulin resistance. *Clin Exp Dermatol* 1984; **9**: 526–31.

(46) Porokeratosis of Mibelli in an immunosuppressed patient

F.M.TATNALL AND I.SARKANY*

*Department of Dermatology, The London Hospital and *Department of Dermatology, The Royal Free Hospital, London*

J.R., male, aged 67 years.

History. The patient developed two lesions on the right upper arm in 1978. In 1971 he had been diagnosed as having chronic active hepatitis and started on prednisolone 30 mg daily, reducing to 15 mg after 3 months. He continued on this treatment until 1977 when he developed osteoporosis. The prednisolone was reduced to 5 mg daily and azathioprine 50 mg was started. In 1985 his liver disease was re-investigated and the diagnosis was changed to alcoholic cirrhosis and the immunosuppression was tailed off.

Examination. There are two lesions on the right upper arm. The medial lesion comprises a scaly plaque with a heaped up rim of scale at the periphery. The lateral lesion has an atrophic centre with a peripheral rim of scale.

Histology. A biopsy from the lateral lesion shows invagination of the epidermis out of which arises a parakeratotic column. Beneath this cornoid lamella the granular layer is absent. A perivascular infiltrate is present in the dermis.

Comment. Immunosuppression is recognized to predispose to the development of porokeratosis and to cause spread of pre-existing lesions.[1] Porokeratosis is believed to represent a clone of mutant cells and immunosuppression may impair the processes which normally contain these cells.

REFERENCE

1 Macmillan AL, Roberts SOB. Porokeratosis of Mibelli after renal transplantation. *Br J Dermatol* 1974; **90:** 45–51.

(47) Porokeratosis of Mibelli following renal transplantation

Drs H.J.Dodd and I.Sarkany

O.L., male, aged 59 years.

History and Examination. He developed chronic renal failure due to chronic glomerulonephritis and underwent a successful renal transplant in 1975. He attended the dermatology

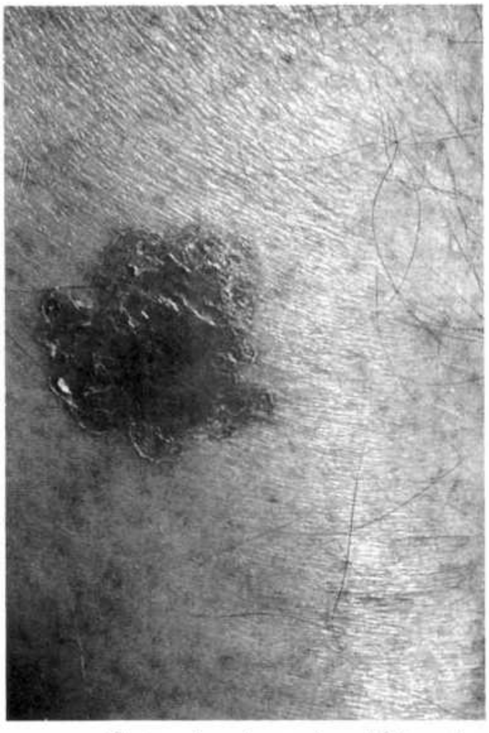

FIGURE 1. Plaque of porokeratosis on left lower leg.

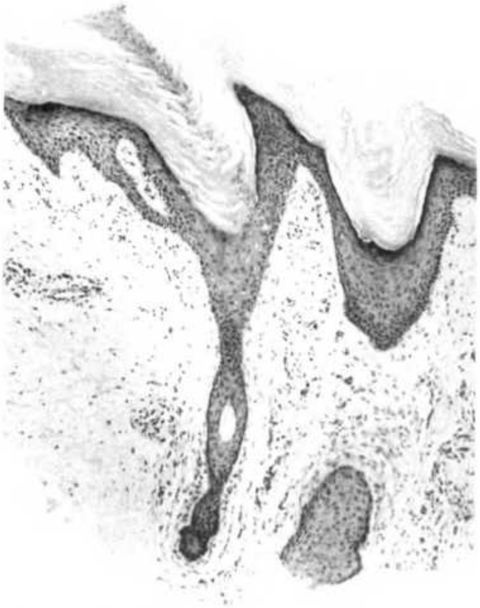

FIGURE 2. Skin biopsy showing typical cornoid lamella.

department in 1987 for treatment of viral warts and actinic keratoses when a plaque of porokeratosis of Mibelli was noted on the left lower leg (Fig. 1). This had been present for 3 years and had grown only slightly in size.

Management. He currently receives azathioprine 75 mg daily, prednisolone 10 mg daily, warfarin 4 mg daily, bumetanide 1 mg daily, spironolactone 50 mg daily and concentrated fish oil capsules 12 g daily.

Little change in the porokeratosis has occurred during the year of follow-up. The viral warts and keratoses have been treated by cryotherapy.

Skin biopsy. There was a small keratin filled furrow in the epidermis. Dyskeratotic cells were present in the epithelium of this furrow and overlying it was a cornoid lamella (Fig. 2). The appearances were consistent with the diagnosis of porokeratosis.

Comment. Porokeratosis of Mibelli is characterized by an abnormality in maturation of keratinocytes, and lesions appear in childhood or adolescence. The onset of porokeratosis late in life has previously been reported in immunosuppressed subjects[1] and it is likely that the immunosuppression is relevant to the development of these lesions. It has been suggested that porokeratosis results from the outward expansion of a clone of abnormal epidermal cells at the expense of normal epidermis.[2] Decreased immunosurveillance has been blamed for the development of several types of tumour in renal transplant recipients and may well allow the development or growth of lesions of porokeratosis.

REFERENCES

1 Tatnall FM, Sarkany I. Porokeratosis of Mibelli in an immunosuppressed patient. *J Roy Soc Med* 1987; **80:** 180–1.

2 Reed RJ, Leone, P. Porokeratosis—A mutant clonal keratosis of the epidermis. *Arch Dermatol* 1979; **101:** 340–7.

(48) Linear porokeratosis

DRS C.BUCKLEY AND I.SARKANY

N.W., female, aged 28 years.

History. This lady first presented at the age of 17 years with a hyperkeratotic lesion with an irregular serpiginous edge on the plantar surface of her right heel. It had been present since childhood and was painful when the skin fissured. The histological findings were consistent with the clinical diagnosis of porokeratosis of Mibelli. She returned in July 1987 with linear brownish keratotic lesions on the right forearm and right groin. There were serpiginous lesions on the right lateral chest and on her face. The groin lesion was pruritic.

Treatment. In 1977 she received a course of Efudix and Retin A gel, neither of which was helpful. Cryotherapy carried out in 1987 improved the groin lesion slightly.

Histology. The section shows a hyperkeratotic lesion traversed by one or two ill-defined narrow tiers of parakeratotic cells. The granular layer is increased.

Comment. It is felt that this rare linear variant of porokeratosis of Mibelli is a distinct one. Squamous and basal cell carcinoma have been reported in all atrophic lesions and consequently patients should be reviewed annually.

REFERENCES

1 Rahbari H, Cordero AA. Linear porokeratosis: A distinctive clinical variant of porokeratosis of Mibelli. *Arch Dermatol* 1974; **109:** 526–8.

2 Sarkany I. Porokeratosis Mibelli with basal cell epithelioma. *Proc Roy Soc Med* 1973; **66:** 435–6.

(49) Tuberculosis verrucosa cutis

DRS D.C.BUCK, H.J.DODD AND I.SARKANY

M.A., male, aged 22 years.

History. This shopkeeper from Pakistan is said to have had a dry warty lesion on his right buttock since childhood. This has gradually increased in size without discomfort. During a visit to Britain the lesion became moist and offensive smelling. He developed a 'boil' in the right groin which, after incision and antibiotics, continued to discharge. There was no personal or family history of tuberculosis.

Examination. On presentation, in January 1988, there was a large, painless, verrucose lesion on the right buttock. Underlying induration extended beyond the margins with hyperpigmentation of adjacent skin. A discharging sinus in the right groin was not associated with lymphadenopathy. Physical examination was otherwise unremarkable.

Investigations. Histology of the lesion from the buttock revealed a dense mixed inflammatory infiltrate, epithelioid granulomata and giant cells within the dermis. There were many plasma cells, lymphocytes and polymorphs. The overlying epidermis was hyperplastic. No foreign material was seen within the granulomata and stains for acid/alcohol fast bacilli and fungi were negative.

Material from both sites failed to reveal acid/alcohol fast bacilli on microscopy. *E. coli*, *Klebsiella pneumoniae*, *Candida* and *Bacteroides spp.* were cultured. X-rays of the chest and right hip were normal as were routine blood tests. Serology for syphilis was negative. Tuberculin testing with 0·5 units of tuberculin diluted 1 : 10,000 was strongly positive.

Treatment. Flucloxacillin given at presentation dried the buttock lesion. Treatment was started with rifampicin 600 mg, isoniazid 300 mg and ethambutol 800 mg daily whilst awaiting culture and sensitivity results.

Comment. Tuberculosis verrucosa cutis is now extremely rare in Britain, occurring after cutaneous inoculation of tubercle bacilli into an immune individual. It is not rare in Asia, where it occurs on the kness and buttocks of boys who play on ground contaminated with tuberculosis sputum.

(50) Dermatitis artefacta and hysterical hemiparesis

DRS H.J.DODD, C.D.CALNAN AND I.SARKANY

I.S., female, aged 59.

History. In 1964 a wart was curetted from her left ring finger and the wound failed to heal. Purulent blistering was said to form and pus exuded for some time. The whole finger became septic and was amputated in March 1966. The surgeon remarked that the whole finger appeared to be affected by masses of warts. The 'sepsis' has spread over the years to involve other limbs. She developed a weakness on the right side in 1975 which led to early retirement. She has needed to wear a leg caliper since. She was married approximately 25 years ago, but was separated from her husband after only 3 weeks.

Examination. There are large offensive smelling, cheesy purulent warty areas of skin over the limbs (Fig. 1). Brown particulate matter is frequently observed when the dressings are removed.

Neurological examination in 1978 revealed a dramatic, bizarre gait and variable loss of the temporal visual field in the right eye only. There was gross fluctuation in power on the right side and complete loss of sensation to pin-prick on the right side with an abrupt mid-line limit. The signs were thought highly suggestive of hysterical conversion.

Investigations. An isotopic brain scan and electro-encephalogram were both normal. All blood

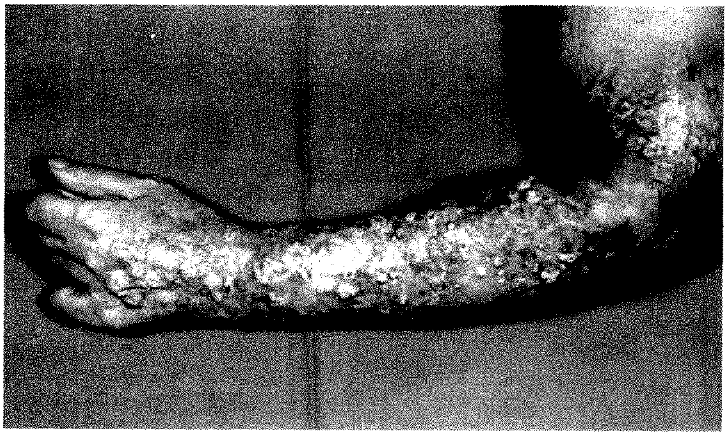

FIGURE 1. Unusual cheesy warty skin over most of the left arm.

investigations have been entirely normal. Faecal streptococci, *Proteus mirabilis* and *Candida* have been isolated at various times from skin swabs.

Management. A period in plaster in 1969 at another hospital led to almost complete clearance of the lesions. The help of our psychiatrists was sought in 1978, but she stopped attending their clinics after she was confronted with the aetiology. The lesions have changed little over the last 10 years and are managed by cleaning and twice-weekly paste bandages.

(51) Ossification in the interosseous membrane of the forearm after etretinate treatment for psoriasis

DRS H.J.DODD, G.DOOTSON AND I.SARKANY

A.M., male, aged 60 years.

History. This man suffered from widespread psoriasis for 20 years and was referred to the Royal Free Hospital in 1983 after we had successfully treated his brother-in-law for psoriasis with etretinate (Tigason®, Roche). He had previously been treated with dithranol as an in-patient on two occasions, but this resulted in only short remissions.

Examination. Extensive plaque type psoriasis with nail changes.

Management. He started etretinate 50 mg daily in January 1983 and continued on a dose of 25–50 mg daily until the drug was stopped in July 1986. During this period his psoriasis was much improved and he required only a limited amount of topical therapy in addition. He noted some soreness of the eyes and lips, but was otherwise asymptomatic. Control of his psoriasis since stopping etretinate has been poor.

Investigations. Liver function tests were monitored throughout the treatment period and remained normal. There was modest elevation of the serum lipids as follows: cholesterol 7·1 mmol/l (normal 3–6·5) and triglycerides 2·5 mmol/l (normal 0·2–1·6).

In July 1986 a limited skeletal survey was performed and the forearms showed ossification in

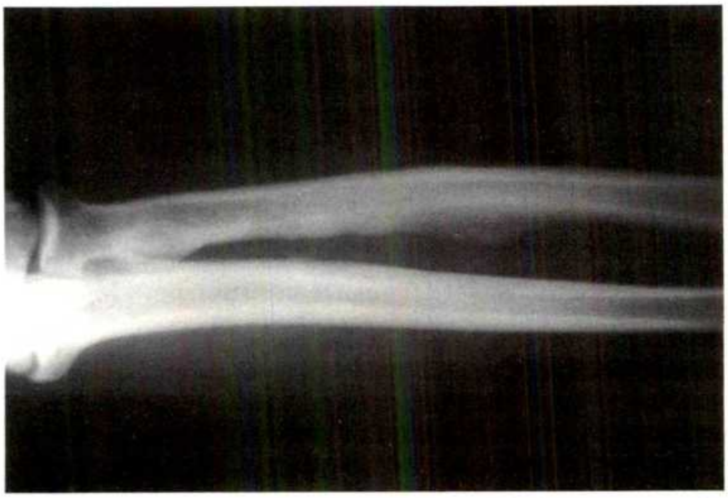

FIGURE 1. X-ray of the forearm showing ossification in the interosseous membrane.

both interosseous membranes. The hands showed no evidence of psoriatic arthritis on X-ray and the cervical, thoracic and lumbar spine showed only minor degenerative changes.

Repeat X-rays of the forearms 1 year after stopping etretinate have shown no progression of the changes.

Comment. It has been known for a long time that hypervitaminosis A can be associated with various abnormalities of bone growth and structure. There have been a number of reports of bony changes with etretinate and most of the abnormalities have been minor and asymptomatic. Unfortunately, there have been few large studies of X-ray changes in patients undergoing treatment with etretinate, but one such study of 35 psoriatics found that 83% had evidence of ligamentous calcification or bony spurring on peripheral joints or both.[1] Some of these patients had also received isotretinoin. The authors suggested that etretinate was more likely to induce changes in the peripheral skeleton rather than DISH (diffuse ideopathic skeletal hyperostosis) syndrome which has been reported with isotretinoin.

One patient receiving etretinate for lamellar ichthyosis who developed pain on pronation and supination later developed a fixed deformity of the forearm associated with ossification of the interosseous membrane.[2] Because of this we had no option but to discontinue treatment after noting the X-ray changes in our patient. Fortunately, there is evidence that increased activity seen on bone scintigraphy during treatment returns to normal after stopping therapy.[3]

Etretinate proved to be a very helpful agent for our patient's psoriasis and his skeletal changes have been entirely asymptomatic.

REFERENCES

1 Di Giovanna JJ, Gerber LN, Helfgotl G, Gantt R.G, Peck GL. Ligamentous calcification and bony spurring after chronic etretinate therapy. *J Invest Dermatol* 1986; **86:** 471.

2 Sillevis-Smitt JH, de Mari F. A serious side-effect of etretinate (Tigason®). *Clin Exp Dermatol* 1984; **9:** 554–6.

3 Graudal C. Extraosseous calcifications during Tigason therapy. *Retinoids Today and Tomorrow* **4:** 13.

(52) Xanthoma disseminatum

DRS G.DOOTSON, J.CLARKE AND I.SARKANY

F.V., male, aged 48 years.

History. This farm worker presented in August 1984 with a 9-month history of pruritic lesions involving the large flexures, scrotum, presacral area, periorbital regions, neck, mouth and ears (Fig. 1).

Examination. There were purple-yellow nodules of varying sizes, some fused into plaques in the above areas. A single lesion was seen on the right pharyngeal wall on indirect laryngoscopy.

Histology. A biopsy showed ill-defined dermal nodules composed of histiocytes with foamy cytoplasm and a diffuse scattering of inflammatory cells. Occasional multinucleate giant cells were present.

Investigations. Cholesterol, triglyceride and electrolyte levels are normal. A skeletal survey showed no evidence of lytic lesions or expansion of the pituitary fossa. Inspection of the airways has failed to show any narrowing.

Treatment. A temporary response was achieved after radiotherapy to limited areas, e.g. presacrally. Dose schedules have ranged from 1200 to 2000 CGy at 40 to 120 kV. A generalized

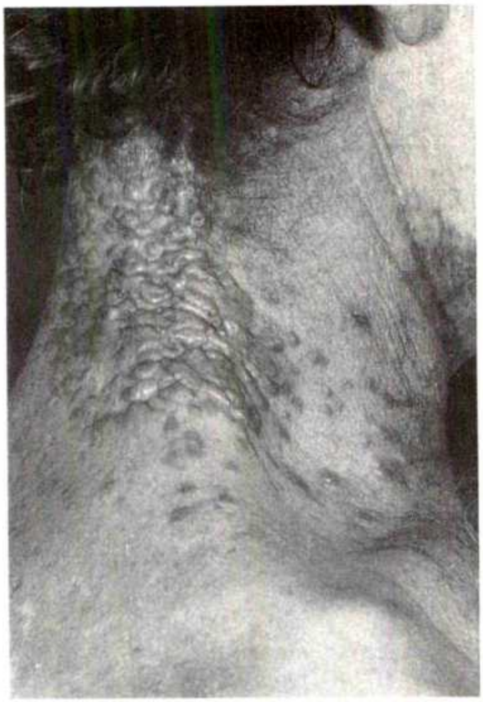

FIGURE 1. Prominent xanthomata on the neck.

reduction in the size of the lesions was also achieved after treatment with the lipid lowering agent, bezafibrate.

Disappointingly, however, this has not been sustained. There has been no response to treatment with chlorambucil and prednisolone.

Comment. The unsatisfactory response to any form of therapy in xanthoma disseminatum is well documented. The patient finds the lesions uncomfortable and they are now interfering with his daily activities. He is being monitored particularly for signs of diabetes insipidus and respiratory obstruction. The temporary improvement with a lipid lowering agent in our patient is difficult to understand, although this effect has been previously documented.[1]

REFERENCE

1 Mishkel MA, Cockshott WP, Nazir DJ, *et al*. Xanthoma disseminatum. Clinical, metabolic, pathologic and radiologic aspects. *Arch Dermatol* 1977; **113:** 1094–100.

(53) Junctional epidermolysis bullosa and squamous cell carcinoma

DRS SUSAN C.PARKER, R.A.J.EADY* AND M.M.BLACK

*Dowling Skin Unit and *The Institute of Dermatology, UMDS, St Thomas' Hospital, London*

M.K., male, aged 56 years.

History. The patient, a Turkish Cypriot, had developed blisters in response to trauma from the age of 7 years, affecting the trunk, and especially the lower legs, with some atrophic scarring but no milia formation. He had had no mucosal lesions and had normal hair, but all his teeth were removed in his thirties. His toenails were mis-shapen, but his finger nails were normal. There was no history of consanguinity nor of other affected family members. A well-differentiated squamous cell carcinoma was excised from clinically normal skin on the dorsum of the right hand in 1976. A persistent ulcer was noted on the anterior aspect of the left lower leg in 1985, but the patient declined excision biopsy until 1986, when a fungating squamous cell carcinoma was treated by below knee amputation. He has also been treated for a transitional cell carcinoma of the bladder.

Examination. There is marked atrophic scarring with ulceration where bullae have occurred on the right lower leg and occasional bullae elsewhere, especially on the amputation stump.

Investigations. Electron microscopy demonstrated separation through the lamina lucida with normal hemidesmosomes. There was reduced staining with the anti-hemidesmosomal antibody GB3. Increased skin fragility was demonstrated by a markedly reduced suction blister time. Direct and indirect immunofluorescence was negative. Taken together these findings confirm the diagnosis of junctional epidermolysis bullosa.

Comment. Squamous cell carcinoma is a well recognized complication of dystrophic epidermolysis bullosa,[1] but we are not aware of its occurrence in junctional forms of the disease. This may be because of the relatively small number of patients who have prolonged survival times.

REFERENCE

1 Reed WB, College J, Francis MJO *et al.* Epidermolysis bullosa dystrophica with epidermal neoplasms. *Arch Dermatol* 1974; **110**: 894–902.

(54) Onset of psoriasis associated with interferon therapy in a patient with hairy cell leukaemia

DRS M.E.MURDOCH AND I.M.LEIGH

The London Hospital, Whitechapel, London

R.L., male, aged 64 years.

History. This patient, an Anglican priest, was diagnosed as having hairy cell leukaemia in March 1983 and a few months later underwent therapeutic splenectomy. Post-operatively, his blood count revealed a persistent neutropenia and the presence of a number of atypical lymphocytes (hairy cells). By January 1985 the hairy cell count had increased and he was commenced on subcutaneous α-interferon therapy, 4 MU thrice weekly. In May 1985, the dose was decreased to 2 MU twice weekly and continued for a further 4 months. He tolerated the treatment well and the hairy cells cleared from the blood, although not from the bone marrow. In March 1986 he was restarted on α-interferon 4 MU thrice weekly for 3 months and then continued on 2 MU twice weekly for a further 11 months.

In May 1986, whilst on interferon therapy, he developed a rash in both axillae which did not respond to prolonged use of topical antifungal agents. In November 1986 he was, therefore, referred to the Skin Department. He had no family history of psoriasis.

Examination. He had erythematous plaques in the flexures which clinically appeared psoriasiform. There was no scalp, nail or joint involvement.

Histology. This was characteristic of psoriasis.

Treatment. He was successfully treated with Haelan C cream.

Comment. α- and β-interferons commonly inhibit the growth of keratinocytes in culture and promote terminal differentiation.[1] Hence α- and β-interferons have been proposed as possible treatments for psoriasis. Despite this, it is possible that our patient's psoriasis was actually triggered by his interferon therapy, as the effect of interferons on psoriasis can be unpredictable.

REFERENCE

1 Yaar M, Karassik RL, Schnipper LE, Gilchrest BA. Effects of alpha and beta interferons on cultured human keratinocytes. *J Invest Dermatol* 1985; **85:** 70–4.

(55) Lichen sclerosus et atrophicus of the palms and soles

DRS K. DALZIEL, J.H.BARTH AND F.WOJNAROWSKA

The Slade Hospital, Oxford

C.H., female, aged 79 years.

History. The patient gave a 20-year history of a pruritic vulval rash. Lesions had appeared in the centre of the palms 10 years ago and on occasions small beads of blood extruded from these. For 5 years, lesions on the feet had made walking uncomfortable.

Examination. There were atrophic white plaques with purpuric elements on the palms and soles, and hypertrophic lichen sclerosus of the vulva.

Histology. Biopsies from the palm and vulva showed hyperkeratosis, epidermal thinning and basal destruction. There was marked oedema and hyalinization of the papillary dermis.

Comment. Lichen sclerosus of the palms and soles is unusual, but has been described in isolation and in association with vulval lesions.[1] The present case suggests that it may represent one cause of 'stigmata'.

REFERENCE

1 Petrozzi JW, Wood MG, Tisa V. Palmar-plantar lichen sclerosus et atrophicus. *Arch Dermatol* 1979; **115:** 884.

(56) Lichen sclerosus et atrophicus and primary biliary cirrhosis

DRS H.J.DODD, I.SARKANY AND O.EPSTEIN

F.B., female, aged 64 years.

History. This patient was first noted to have abnormal liver function in 1980 whilst undergoing investigations for fatigue and suspected angina. In 1981 she noted patches of shiny wrinkled skin on the left breast and left groin, as well as on the vulval and perianal skin. She has been troubled by generalized pruritus.

Examination. She was found to have hepatomegaly, but no jaundice, splenomegaly or ascites. Areas of pale atrophic wrinkled skin were present on the left breast, lower abdomen and thigh. Similar changes were found on the vulval and perianal skin.

Investigations. Liver function tests: Alkaline phosphatase was 665 IU/l (normal 35–130), aspartate transaminase 86 IU/l (normal 5–40) and γ-glutamyl transpeptidase 840 IU/l (normal 10–40). The serum bilirubin, albumin and total protein, and the prothrombin time were all normal. Antimitochondrial antibodies were present to a titre of greater than 1:40.

Histology. A liver biopsy taken in 1980 showed that although the lobular architecture was preserved, the portal tracts were expanded and fibrotic. There was bile duct damage and a portal inflammatory infiltrate included plasma cells, eosinophils, lymphoid aggregates and collections of histiocytes. The latter surrounded damaged bile ducts. The appearances were highly suggestive of early primary biliary cirrhosis.

A skin biopsy taken in 1982 showed an atrophic epidermis with prominent hyperkeratosis, loss of rete ridges and focal basal cell liquefaction. There was a scattered chronic inflammatory cell infiltrate in the upper dermis associated with pale staining collagen fibres which were fragmented and in places homogenized. The appearances were those of lichen sclerosus et atrophicus.

Treatment. The use of 0·1% hydrocortisone 17-butyrate cream (Locoid®, Brocades) has been helpful. For her liver disease she is receiving monthly injections of vitamins A, D and K together with colestipol 2 sachets daily and microcrystalline hydroxyapatite compound (MCHC) tablets.

Comment. Primary biliary cirrhosis has been previously reported in association with lichen sclerosus et atrophicus,[1] but this is rare and possibly coincidental. In a study of 250 females with lichen sclerosus et atrophicus, Meyrick Thomas *et al.*[2] found only four patients with antimitochondrial antibodies and none of these had developed auto-immune liver disease. In contrast, there have been many reports of lichen planus and scleroderma occurring with primary biliary cirrhosis and it is thought relevant that features of all three disorders may occur in chronic graft-versus-host disease.[3]

REFERENCES

1 Lavery, HA, Pinkerton, JHM, Callender M. The association of lichen sclerosus et atrophicus and primary biliary cirrhosis. *Br J Dermatol* 1985; **112**: 729–30.

2 Meyrick Thomas RH, Ridley CM, McGibbon DH, Black MM. Lichen sclerosus et atrophicus and auto-immunity in 250 women. *Br J Dermatol* 1985; **113**: (Suppl. 29) 34; 28–9.

3 Sarkany I. In. *Dermatology in General Medicine*, 3rd ed. New York: McGraw-Hill 1987; 1954–6

(57) Localized morphoea at site of previous radiotherapy

DRS S.M.NEILL, J.J.NICHOLL, I.W.F.HANHAM AND R.C.D.STAUGHTON

Westminster Hospital, London

P.B., female, aged 62 years.

History. In 1983 this patient had a local excision of an adenocarcinoma of the right breast followed by radiotherapy. In 1985 metastasis was suspected when subcutaneous nodules developed in the upper half of the right breast, but histologically the lesions were benign. Further nodules developed in 1986 and by 1987 the overlying skin had become tethered with a shiny waxy appearance.

Examination. Two plaques of morphoea were noted localized to the site of previous irradiation. The larger plaque involved the upper segment of the right breast with a smaller area on the anterior chest wall above the breast.

Histology. Epidermal thinning with a periadnexal and perivascular mixed inflammatory cell infiltrate.

Progress. Chloroquine 200 mg on alternate days has produced clinical improvement.

Comment. Trauma has been implicated as a precipitating factor in localized morphoea. A previous case following radiotherapy for breast carcinoma has been noted.[1] Similar localization of lichen sclerosus following radiotherapy has been described.[2]

REFERENCES

1 Rowell NR. Localized morphoea. In: *Textbook of Dermatology* (Rook A, Wilkinson DS, Ebling FJG *et al.* eds). Oxford: Blackwell Scientific Publications, 1986; 1334–5.

2 Yates VM, King CM, Dave VK. Lichen sclerosus et atrophicus following radiation therapy. *Arch Dermatol* 1985; **121:** 1044–7.

(58) Mycosis fungoides and parapsoriasis lichenoides (parakeratosis variegata)

S.M.Neill, C.C.Harland, C.M.E.Rowland Payne, J.Mc.L.Singleton* and R.C.D.Staughton

*Westminster Hospital and *Queen Mary's Hospital, London*

M.A., female, aged 64 years.

History. This Iranian lady has a 30-year history of a pigmented pruritic eruption on the trunk and limbs. Previous treatment has included topical steroids and PUVA. She was referred for

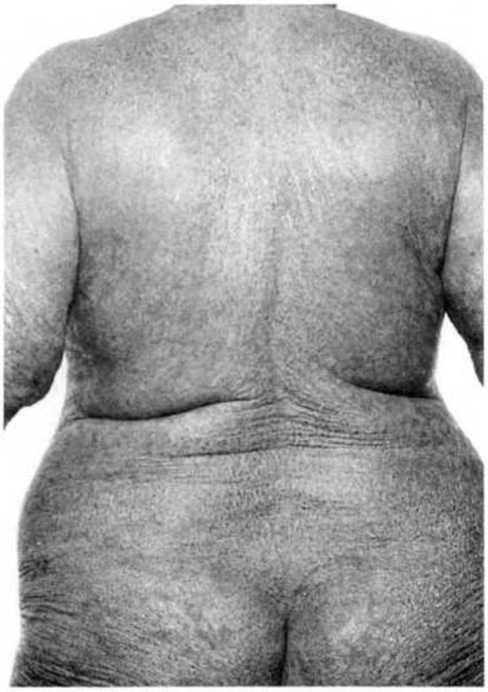

FIGURE 1. Widespread lesions of parapsoriasis lichenoides.

dermatological opinion in 1987 by the surgical department who were investigating her for a probable hydatid cyst of the liver.

Examination. There was a widespread hyperpigmented eruption consisting of shiny, flat topped papules many of which had coalesced forming a retiform pattern, predominantly affecting the trunk and proximal extremities (Fig. 1). Poikilodermatous changes were prominent.

Investigations. An abdominal X-ray and ultrasound examination show a large peripherally calcified shadow in the liver. An abdominal CT scan suggests that the para-aortic nodes are enlarged. An hydatid ELISA was positive.

Histology. This has varied from a non-specific dermal lymphocytic infiltrate without evidence of epidermotropism, to changes consistent with mycosis fungoides.

Progress. She has responded well to further PUVA therapy.

ACKNOWLEDGMENT

Dr C.D.Calnan presented this patient to a meeting at the St. John's Dermatological Society in February 1987.

(59) Case for diagnosis: possible Cowden's disease (multiple hamartoma and neoplasia syndrome)

DRS J.ASHWORTH, P.W.M.COPEMAN, S.M.BREATHNACH AND J.J.CREAM

Charing Cross and Westminster Medical School

S.D., female, aged 19 years.

History. Since childhood the patient was noted to have short, broad fingers; her mother had similar abnormalities. Congenitally shortened Achilles tendons were corrected, orthopaedically, when the patient was aged 3 and again at 15 years. At the age of 12, asymptomatic papules

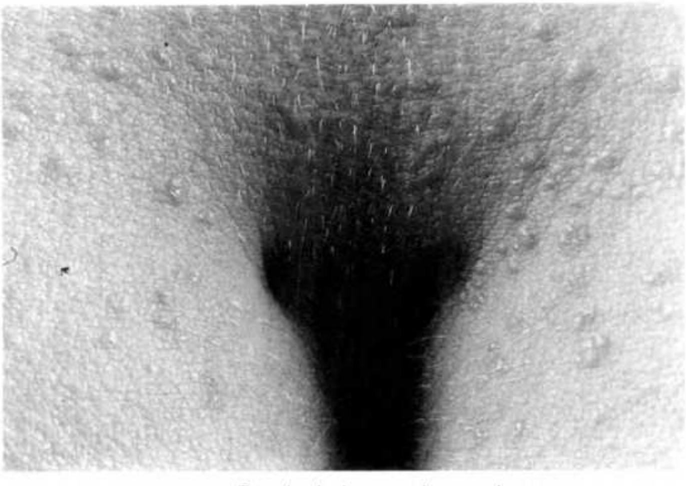

FIGURE 1. Papular lesions on the sacral area.

appeared, initially affecting the periorbital skin, but gradually extending to involve the forehead, cheeks, trunk, buttocks, upper thighs and vulva. Her main complaint was of vulval irritation.

Examination. There are scattered, skin coloured papules on the face, lower back and buttocks (Fig. 1). Vulval involvement consists of multiple papules becoming confluent and giving a cobblestone appearance. The fingers are shortened with thickened terminal phalanges and there is a simian crease. There is ocular hypertelorism, abnormal dentition with cramping of the lower teeth and a high arched palate.

Histology. Biopsy of a lesion from the neck showed a syringoma.

Comment. This case is difficult to classify but has several of the features of Cowden's disease, a rare disorder with autosomal dominant inheritance characterized by multiple facial papules (usually trichilemmomas) in association with mucosal papillomatosis (histologically fibromata). A wide variety of other abnormalities has also been described, the most important of which are palmo-plantar keratoses, keratoses involving the dorsa of hands and feet, a high arched palate and adenoid facies.

The importance of this rare condition lies in its association with multisystem hamartomata and with internal malignancy (especially in females) particularly of the breast (36%) and thyroid (12%).

Syringomata are, however, not a recognized feature of Cowden's disease.

REFERENCES

1 Salem, OS, Steck, WD. Cowden's disease (multiple hamartoma and neoplasia syndrome). *J Am Acad Dermatol* 1983; **8:** 686–96.
2 Brownstein MH, Mehregan AR, Bikowski JB *et al.* The dermatopathology of Cowden's syndrome. *Br J Dermatol* 1979; **100:** 667–673.

(60) Basal cell naevus syndrome (lacking basal cell carcinomata)

Drs J.N.W.N.Barker and D.M.MacDonald

Guy's Hospital, London

D.B., male, aged 18 years.

History. Seven years ago this patient presented with multiple painful jaw cysts. As an infant he suffered from hydrocephaly, which resolved spontaneously. There was no relevant family history.

Examination. Several features typical of the basal cell naevus syndrome were present including hypertelorism, palmo-plantar pits and multiple epidermoid cysts. There was no clinical evidence of basal cell carcinomata.

Examination of both parents and the patient's brother revealed no abnormality.

Histology. The excised jaw cysts proved, histologically, to be odontogenic keratocysts. Biopsy of a palmar pit failed to reveal a basal cell carcinoma at the base. There were no abnormal features associated with an excised epidermoid cyst.

Radiology. An orthopantomogram demonstrated multiple jaw cysts, one with a tooth within the cavity, typical of odontogenic keratocysts (Fig. 1). A chest X-ray revealed bifid ribs and a skull X-ray showed calcification within the falx cerebri.

Comment. Despite the presence of multiple features of the basal cell naevus syndrome, it is of

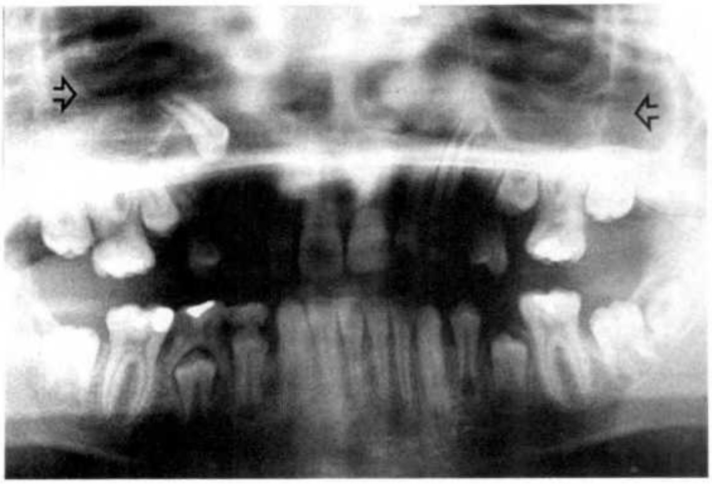

FIGURE 1. Orthopantomogram demonstrating multiple odontogenic keratocysts (arrows).

interest that, to date, no basal cell carcinomata have developed. These most commonly develop at puberty. Hydrocephaly is a rare association of this syndrome.

REFERENCE

1 Gutierrez MM, Mora RG. Nevoid basal cell carcinoma syndrome. *J Am Acad Dermatol* 1986; **15:** 1023–30.

(61) Ulerythema ophryogenes in Noonan's syndrome

DRS A.C.MARKEY, M.J.TIDMAN, D.E.SHARVILL AND R.S.WELLS

Guy's Hospital, London

M.J., male, aged 16 years.

History. This boy presented with ulerythema ophryogenes (keratosis pilaris atrophicans faciei) at 9 years of age. There was typical follicular hyperkeratosis of the preauricular regions and the outer aspect of the arms and legs, and alopecia of the eyebrows. Features of Noonan's syndrome were also noted, including relatively short stature, undescended testes, curly hair and hypertelorism. A mild degree of pulmonary stenosis was also present and subsequently he has developed numerous melanocytic naevi.

Investigations. He is now pubertal and is of normal height and above average intelligence. Pubertal hair has appeared, although secondary sexual hair, elsewhere, is still lacking. The extent of the ulerythema has not changed, although there has been a recent significant growth of eyebrow hair (Fig. 1).

Comment. This case illustrates the association between Noonan's syndrome and ulerythema ophryogenes,[1,2] and it is of interest that this cutaneous disorder may be the presenting feature of Noonan's syndrome.

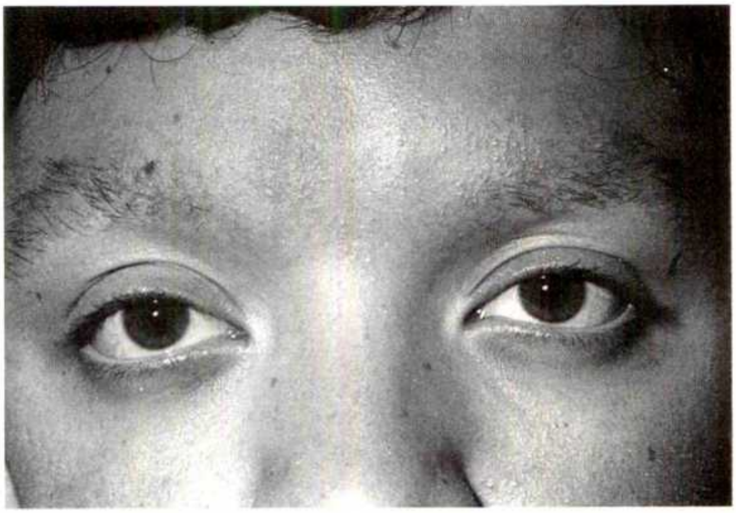

FIGURE 1. Ulerythema ophryogenes in Noonan's syndrome.

REFERENCES

1 Wyre HW. Cutaneous manifestations of Noonan's syndrome. *Arch Dermatol* 1978; **114:** 929–30.

2 Pierini DO, Pierini AM. Keratosis pilaris atrophicans faciei (ulerythema ophryogenes): a cutaneous marker in the Noonan syndrome. *Br J Dermatol* 1979; **100:** 409–16.

(62) Multiple lentigines syndrome

DRS P.D.L.MAURICE AND S.M.BREATHNACH

Charing Cross Hospital, London

C.G., female, aged 31 years.

History. This patient has had profuse pigmented lesions of the skin since early childhood. At the age of 12 years, she was investigated for an asymptomatic heart murmur; right heart catheterization and angiography showed mild pulmonary stenosis. At the age of 24 years, a granular cell myoblastoma was excised from the left breast, and five years later a neurofibroma was removed from the right temple. There was no relevant family history.

Examination. She was noted to have profuse lentigines, some of which were up to 1 cm in diameter and showed a speckled pigmentation. These were most numerous on the limbs and the face and they extended onto the scalp, but spared the mucosae (Fig. 1). Her height was below the third centile and she showed hypertelorism. There was a soft ejection systolic murmur at the left sternal edge.

Investigations. An ECG showed right ventricular strain, right axis deviation and pointed P-waves. Biopsy of one of the pigmented lesions showed a lentigo.

Comment. This patient has most of the cardinal features of multiple lentigines syndrome, also known as 'LEOPARD syndrome' (Lentigines, multiple; electrocardiographic conduction defects; ocular hypertelorism; pulmonary stenosis; abnormalities of genitalia; retardation of growth; deafness, sensorineural).[1,2] It is interesting that she had a granular cell myoblastoma, since this tumour has been rarely described in LEOPARD syndrome.[2] The tumour is believed

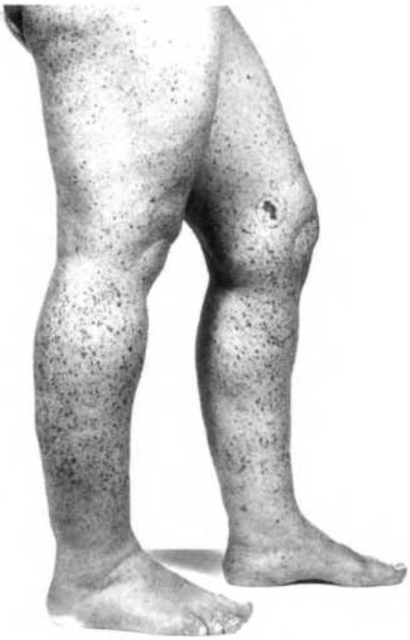

FIGURE 1. Profuse lentigines on the legs.

to arise from Schwann cells which, like melanocytes, are derived from the neural crest. LEOPARD syndrome is thought to be due to a genetically-determined dysfunction of pigmentary and other elements of neural crest origin.[3]

REFERENCES

1 Gorlin RJ, Anderson RC, Blaw M. Multiple lentigines syndrome. *Am J Dis Child* 1969; **117:** 652–62.
2 Voron DA, Hatfield HH, Kalkhoff RK. Multiple lentigines syndrome. *Am J Med* 1976; **60:** 447–56.
3 Polani PE, Moynahan EJ. Progressive cardiomyopathic lentiginosis. *Q J Med* 1972; **41:** 205–25.

(63) Sarcoidosis of the scalp presenting as scarring alopecia

DRS P.D.L.MAURICE AND S.K.GOOLAMALI

Central Middlesex Hospital, London

I.M., female, aged 58 years.

History. This Jamaican lady has had bald patches in her scalp for the last 23 years. Apart from glaucoma diagnosed in 1981, there was no relevant medical history.

Examination. There were large areas of scarring with induration, showing varying degrees of erythema and scaling, mainly over the occiput and extending onto the back of the neck and the ears. There were some smaller erythematous plaques on the arms.

Histology. Biopsies from lesions in the scalp and on the arm both showed the presence of dermal non-caseating granulomata with Langhans giant cells and hyperplasia of the overlying epidermis. Stains for acid-fast bacilli and fungi were negative. Direct immunofluorescence was negative. The Kveim test was positive.

Investigations. Culture of material from the scalp lesions for fungi was negative. A full blood count, ESR, liver function tests and serum calcium were normal. Anti-nuclear factor and syphilis serology were negative. Serum angiotensin converting enzyme was within the normal range. The Mantoux test was negative at 1 : 1000. A chest X-ray showed a calcified primary complex only, and hand X-rays were normal.

Comment. This is an unusual presentation for sarcoidosis, but one which is recognized, predominantly in black females.[1]

REFERENCE

1 Golitz LE, Shapiro L, Hurwitz E, Stritzler R. Cicatricial alopecia of sarcoidosis. *Arch Dermatol* 1973; **107:** 758–60.

(64) Necrobiosis lipoidica and sarcoidosis

DRS H.J.DODD AND I.SARKANY

A.P., female, aged 48 years.

History and examination. In 1975 a routine pre-operative chest X-ray showed bilateral hilar lymphadenopathy and patchy shadowing in the lung fields compatible with sarcoidosis. At the

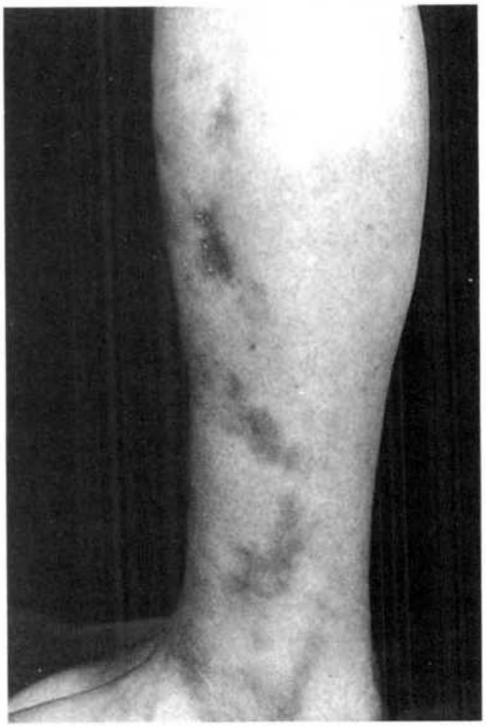

FIGURE 1. Typical appearances of necrobiosis lipoidica on the leg.

time, yellow atrophic areas of necrobiosis lipoidica were noted on both shins (Fig. 1). In 1977, she developed pea-sized nodules of cutaneous sarcoid over the left arm, forehead and back. These responded to systemic steroids, but the necrobiosis remained unchanged.

Investigations. A glucose tolerance test was normal. The chest X-ray showed enlargement of both hilar shadows and there were infiltrative changes in both upper lung fields compatible with sarcoidosis. Pulmonary function tests showed reduced transfer of carbon monoxide.

Histology. A biopsy from her shin showed changes consistent with necrobiosis lipoidica. The epidermis was atrophic. The dermis showed extensive collagen degeneration and contained numerous poorly defined granulomata, some of them containing giant cells.

Biopsies from her neck and back showed numerous well-circumscribed epithelioid granulomata with scattered giant cells and no evidence of caseation. The changes were consistent with cutaneous sarcoidosis.

Management. Her pulmonary and cutaneous sarcoid responded to systemic steroids with an improvement in gas transfer and resolution of the skin lesions. Her necrobiosis lipoidica has been helped by 0·05% fluocinonide cream (Metosyn®, Stuart). She has recently started a trial of methotrexate 10 mg weekly.

Comment. The co-existence of sarcoidosis and necrobiosis lipoidica may be coincidental, but the association has been reported before[1] and granuloma annulare has also been described in association with sarcoidosis.[2]

REFERENCES

1 Graham-Brown RAC, Shuttleworth D, Sarkany I. Co-existence of sarcoidosis and necrobiosis of the legs—A report of two cases. *Clin Exp Dermatol* 1985; **10**: 274–8.

2 Umbert P, Winkelmann RK. Granuloma annulare and sarcoidosis. *Br J Dermatol* 1977; **97**: 481–6.

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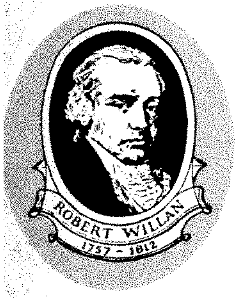

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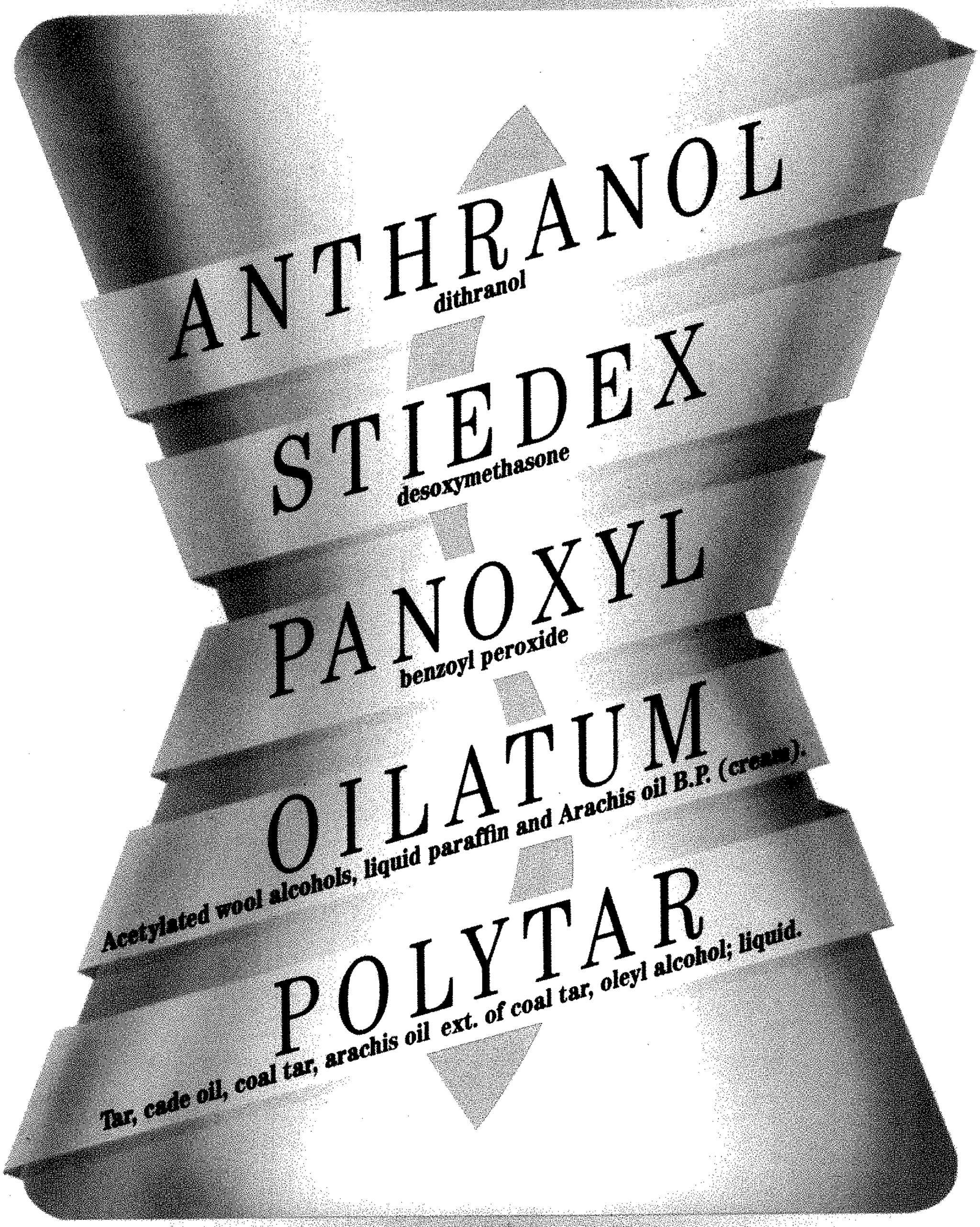
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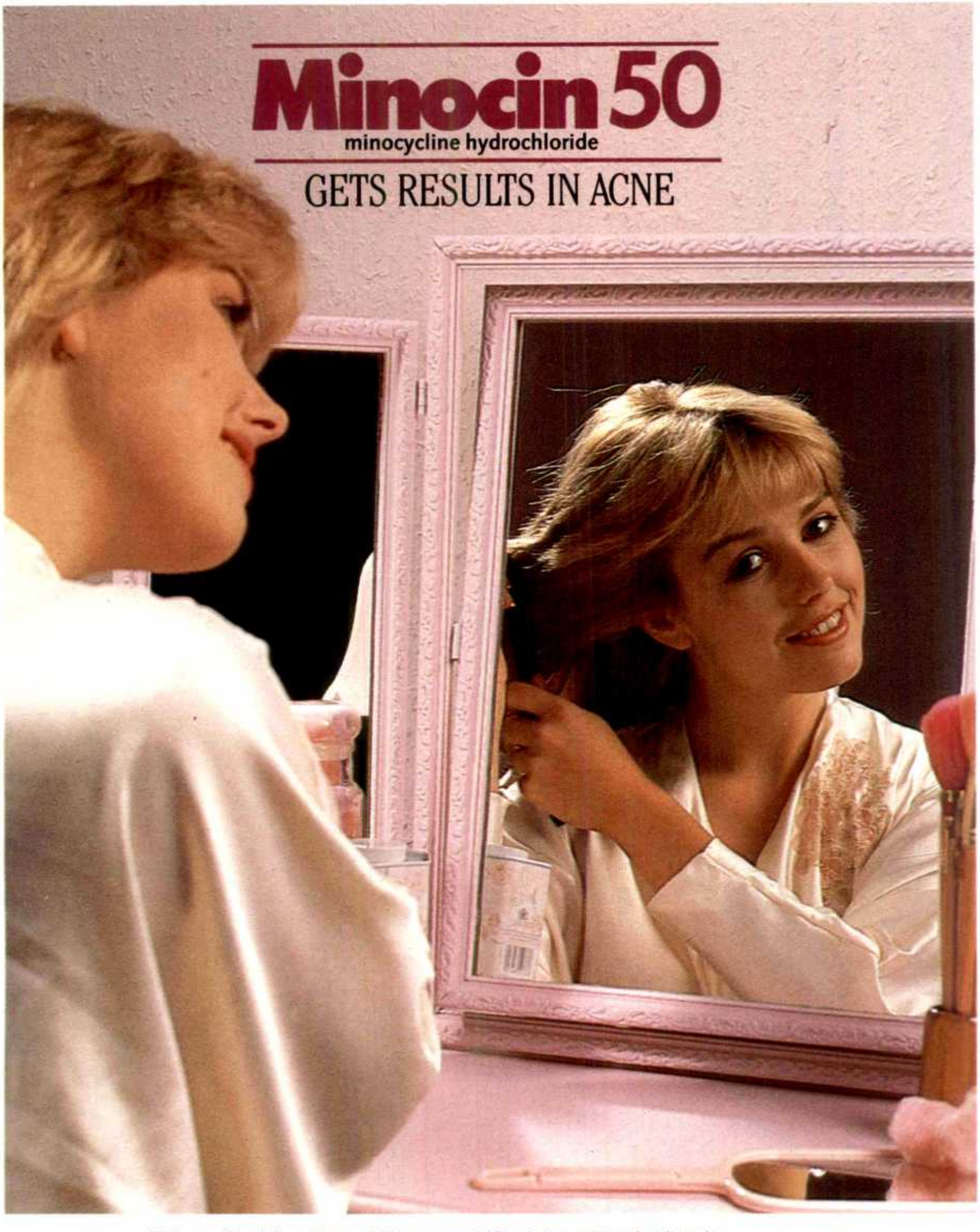

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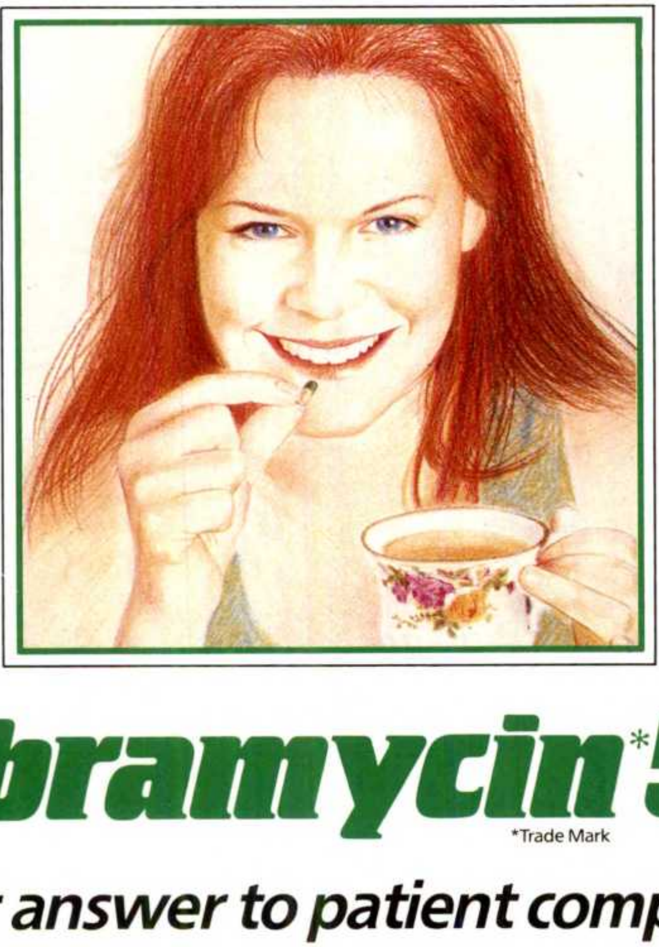

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REFERENCES

1. Mead JF & Fulco AJ 1976 The unsaturated and polyunsaturated fatty acids in health and disease. C C Thomas, Springfield, USA.
2. Rivers JP Br Med Bull 1981; **37**: 59-64.
3. Manku MS *et al* Br J Dermatol 1984; **110**: 643-648.
4. Ziboh VA & Chapkin RS. Prog Lipid Res 1988; **27**: 81-105.
5. Horrobin DF. J Am Acad Dermatol, in press.
6. Stewart JCM *et al*. Submitted.
7. Wright S & Burton JL. The Lancet 1982; 2: 1120-1122.
8. Schalin-Karrila *et al*. Br J Dermatol 1987; **117**: 11-19.
9. Morse PF *et al*. In Press.

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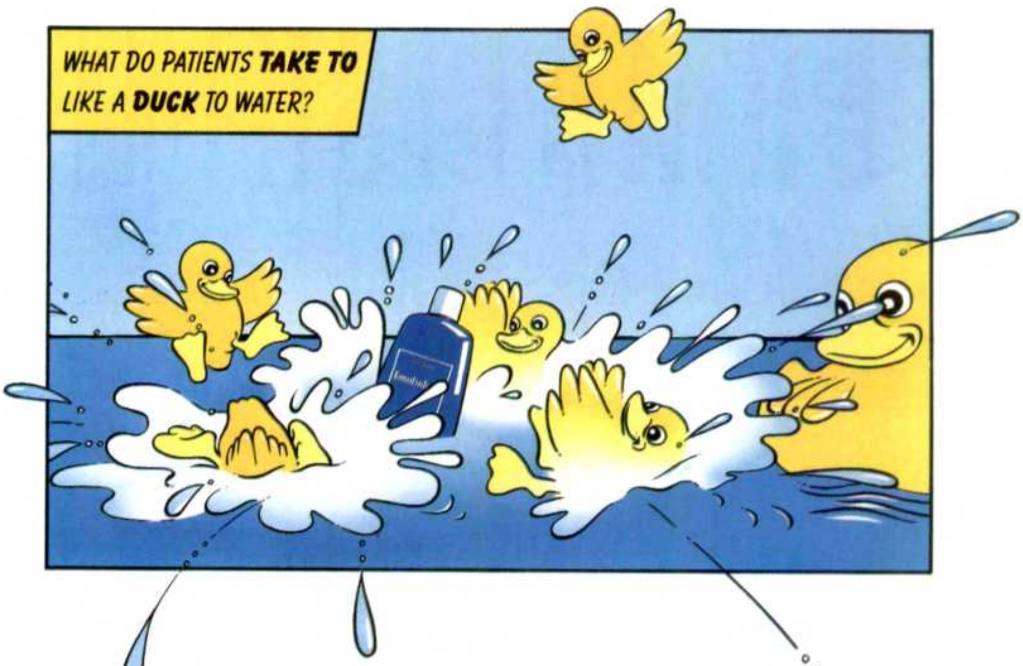
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British Journal of Dermatology (1989) **120**, 1–7.

Gamma interferon induces different keratinocyte cellular patterns of expression of HLA-DR and DQ and intercellular adhesion molecule-1 (ICAM-1) antigens

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Accepted for publication 30 June 1988

SUMMARY

With indirect immunofluorescence techniques we demonstrated that recombinant gamma-interferon induced the expression of the class II antigens HLA-DR and HLA-DQ as well as intercellular adhesion molecule-1 (ICAM-1) on normal, cultured human keratinocytes grown in low-calcium, serum-free medium. Each antigen displayed a distinctive cellular staining pattern. HLA-DR was strongly localized to perinuclear zones with intense cell surface expression; HLA-DQ displayed a perinuclear accentuation, but with minimal cell surface staining, and ICAM-1 was strongly expressed in a diffuse cytoplasmic pattern with intense cell surface expression. Keratinocytes grown in medium supplemented with 10% fetal calf serum underwent differentiation, with a diminished expression of all three antigens as compared to those grown in low-calcium, serum-free medium.

These results confirm that gamma interferon can differentially regulate HLA-DR and HLA-DQ expression; that there are probably different biochemical metabolic pathways by which these three molecules are expressed on keratinocytes, and that the expression is also a function of the degree of keratinocyte differentiation. The strong cell surface expression of ICAM-1 is suggested to be of major importance as the recognition molecule, by which T cells bind to gamma interferon exposed keratinocytes, and suggests an integral role for this molecule in epidermal lymphocyte trafficking.

The human class II major histocompatibility complex (MHC) antigens are coded for by three distinct loci on chromosome six: HLA-DR, HLA-DQ and HLA-DP.[1] Unlike class I MHC

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antigens, which are ubiquitously expressed, class II antigens have a limited cellular distribution on human nucleated cells.[2,3] HLA-DR expression on macrophages is necessary for antigen to be presented to T lymphocytes,[4] and for induction of cytolytic T lymphocyte activity,[5] and is involved in the autologous mixed lymphocyte reaction (AMLR).[6] Much less is known about the functional significance of HLA-DQ. However, it has similar effects to HLA-DR and Gonwa *et al.*[7] have shown it to be required for antigen presentation in the AMLR. In normal skin, both HLA-DR and HLA-DQ are found on the same cells, namely vascular endothelium,[8] Langerhans cells[9] and the acrosyringial epithelium.[10] Under normal circumstances neither antigen is expressed by keratinocytes (KC).[11] In diseased skin, particularly the inflammatory dermatoses, HLA-DR is commonly expressed by KC[12] and has been related to the presence of intra-epidermal lymphocytes.[13] HLA-DQ expression by KC is infrequent although it has been observed in cutaneous T cell lymphoma, lichen planus,[14] *Borrelia*-induced skin disease,[15] and allergic and irritant contact dermatitis.[16]

Gamma interferon IFN-γ, induces HLA-DR expression on normal cultured human KC[17] and direct intradermal injection of IFN-γ will induce the appearance of HLA-DR on the overlying epidermal KC.[18] However, IFN-γ has not been demonstrated to induce HLA-DQ on normal cultured human KC[19] or on KCs in a short-term organ culture system.[11,20]

Intercellular adhesion molecule-1 (ICAM-1) is a cell surface glycoprotein of molecular weight 90–114 kd expressed by both haemopoeitic and non-haemopoeitic cells.[21,22] It is involved in the regulation of lymphocyte–cell adherence mediated by lymphocyte function-associated antigen-1 (LFA-1) which is a ligand for ICAM-1.[23] The adherence of resting and activated autologous and allogeneic T lymphocytes to IFN-γ treated KC is blocked by anti-LFA-1 antibodies[24,25] or anti-ICAM-1 antibody,[26] thus implying a role for ICAM-1 in lymphocyte–KC interactions in the inflammatory dermatoses.

We have investigated the cellular distribution patterns of HLA-DR, HLA-DQ and ICAM-1 in normal human cultured KC in serum-free and serum-supplemented medium and the effect of IFN-γ on the expression of these antigens.

METHODS

Preparation of keratinocyte cultures

Samples of normal human skin were obtained from patients undergoing cosmetic surgery and single cell suspensions prepared according to the method of Liu and Karasek.[27] Small, round, viable cells were seeded onto and grown in either 10 cm diameter Petri dishes (Lux Flow Laboratories, Inc) or Lab-Tek chamber slides (Miles Scientific, Inverhill, IL, U.S.A.) in either a serum-free KC growth medium containing low calcium[28] (KGM, Clonetics, CO., San Diego, CA, U.S.A.) or Dulbecco's modified Eagle's medium (DMEM, Gibco Laboratories, Grand Island, NY) supplemented with 10% heat inactivated fetal calf serum (FCS, Gibco). Cells were maintained in a humidified incubator in 5% Co_2/95% air at 37°C and used after passage 3.

Recombinant IFN-γ (specific activity $= 1{\cdot}7 \times 10^7$ U/mg; kindly provided by Dr M.Shepard, Genentech Inc., South Francisco, CA, U.S.A.) was used at a final concentration of 1200 U/ml and added to dishes or slides containing sub-confluent KC, after which incubation was continued for 3 days at 37°C.

Staining of keratinocyte for HLA-DR, HLA-DQ and ICAM-1

KC in Lab-Tek chamber slides were either fixed for 15 min in acetone at 4°C to permeabilize the cell membrane prior to staining, or were left unfixed. The KC monolayers were incubated for 25

TABLE 1. Monoclonal antibodies used in the study

Antibody	Specificity	Isotype	Source
TS1.16	HLA-DR	$\gamma 1$	Dr C.Clayberger, Stanford University U.S.A.
Leu 10	HLA-DQ	$\gamma 1$	Becton Dickinson, Mountain View, CA, U.S.A.
RR-11	ICAM-1	$\gamma 1$	Dr T.Springer, Dana Farber Cancer Institute, Boston, MA, U.S.A.
OKT8	CD8	$\gamma 1$	Ortho Diagnostics, Raritan, NJ, U.S.A.

min on ice with 10μg/ml of the panel of monoclonal antibodies listed in Table 1. OKT8 was used as an isotype irrelevant control antibody. The cells were washed and then stained with 1 μg of fluorescein isothiocyanate (FITC)-conjugated anti-mouse IgG (Tago Inc., Burlingame, CA, U.S.A.). In order to investigate the possibility of anti-HLA-DQ antibody binding to HLA-DR antigen, which may have been altered after acetone fixation,[29] KC were stained directly with FITC-conjugated Leu 10 (Becton Dickinson, Mountain View, CA, U.S.A.) for 25 min on ice and compared with KCs pre-incubated with TS 1·16 for 1 h on ice prior to staining with FITC-conjugated Leu 10. The cells were examined using an Olympus BH-2 fluorescence microscope.

Cultured KC were trypsinized using 0·03% trypsin/0·01% EDTA, an aliquot of 10^6 KC was stained as described above, and staining intensity and numbers of cells stained determined using fluorescence activated cell sorter (Epics 541: Coulter Corp), as previously described.[17]

RESULTS

Induction of HLA-DR, HLA-DQ and ICAM-1 antigen expression by IFN-γ

Normal human KC grown in KGM in the absence of IFN-γ did not express HLA-DR, HLA-DQ or ICAM-1. However, the addition of IFN-γ (1200 U/ml) induced distinct patterns of cellular expression for all three antigens. The staining patterns described combine the cell surface staining seen in the absence of fixation or permeabilization, and the intracellular staining seen after acetone treatment.

HLA-DR was localized to the perinuclear zone with intense cell suface expression (Fig. 1), as has been observed previously.[30] HLA-DQ showed perinuclear localization, but with minimal cell surface staining (Fig. 2). ICAM-1 was strongly expressed in a diffuse cytoplasmic pattern with intense cell surface expression (Fig. 3). Using FITC-conjugated Leu 10 (anti-HLA-DQ) there was no observable inhibition of staining when the KC were pre-incubated with TS 1·16, suggesting that the Leu 10 antibody was not binding to HLA-DR antigen (data not shown). This was also confirmed by the difference in the intensity of cell-surface staining between HLA-DR and HLA-DQ.

Effect of serum-supplemented medium on the IFN-γ-induced expression of HLA-DR, HLA-DQ and ICAM-1

All three antigens were expressed less strongly on KC cultured DMEM + 10% FCS than on cells cultured in KGM. The cell surface expression of ICAM-1 and HLA-DR was similar to that seen on KC cultured in KGM, but the intracellular staining was markedly reduced (data

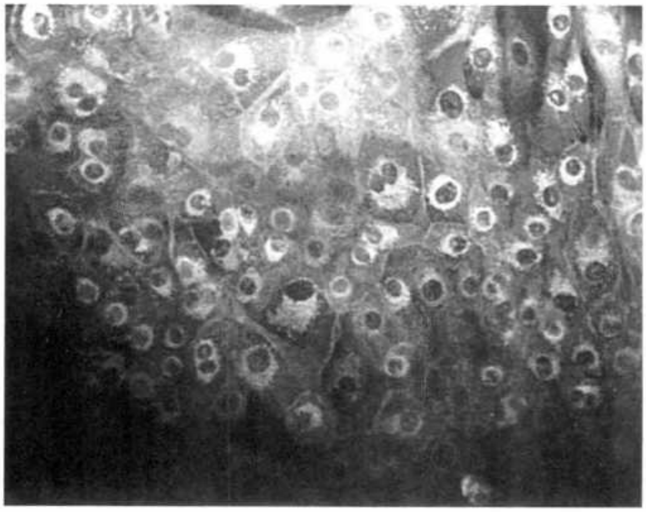

FIGURE 1. IFN-γ-induced expression of HLA-DR on acetone-fixed keratinocytes showing perinuclear and intense cell surface staining (original × 150).

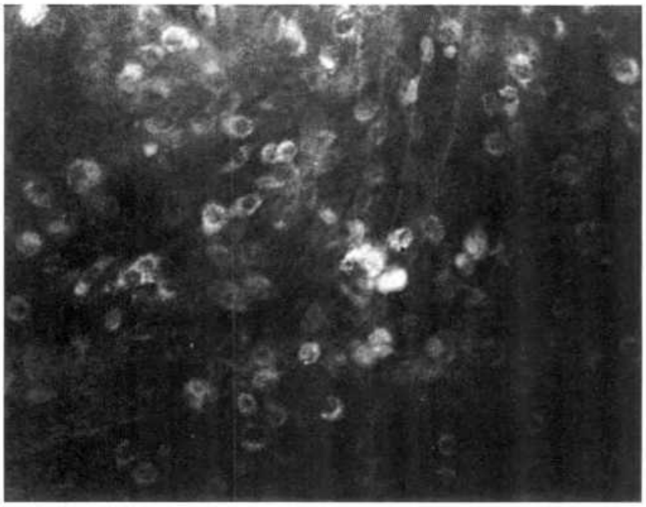

FIGURE 2. IFN-γ-induced expression of HLA-DQ on acetone-fixed keratinocytes showing perinuclear and minimal cell surface staining (original × 150).

not shown). There was no cell surface expression of HLA-DQ on KC cultured in DMEM + 10% FCS and the intracellular staining was also markedly reduced.

Intensity of cell surface expression of HLA-DR, HLA-DQ and ICAM-1 antigens using FACS analysis

After subtraction of OKT8 staining (which was used as the IgG_1 isotype control) background values, the mean channel readings for the three antigens studied were: HLA-DQ: 18, HLA-DR: 52 and ICAM-1: 79.

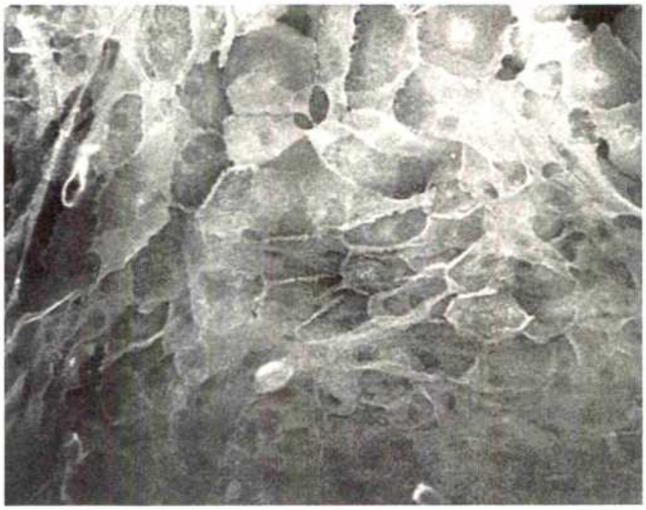

FIGURE 3. IFN-γ-induced expression of ICAM-1 on acetone-fixed keratinocytes showing intense cell surface and diffuse cytoplasmic staining (original × 150).

These values are for KC grown in KGM supplemented with IFN-γ (1200 U/ml) for 3 days. In the absence of IFN-γ, the mean channel readings were 0 for all three antigens, as well as for the OKT8 stained KC. It is important to use isotype controls because we have previously observed that IFN-γ induces expression of Fc receptors in cultured KC.[19] It should also be noted that the mean channel readings presented are on a log scale; thus the values represent a considerable degree of fluorescence intensity.

DISCUSSION

This study has demonstrated the ability of IFN-γ to induce contrasting and distinctive cell surface and intracellular expression of HLA-DR, HLA-DQ and ICAM-1 antigens in keratinocytes. The expression of these antigens was diminished by growing the KC in medium supplemented with serum. The induction of HLA-DQ by IFN-γ is of interest as Basham *et al.*[19] in a previous study, were unable to demonstrate this antigen on normal human KC. This disparity can be explained by the fact that they cultured KC in DMEM + 10% FCS which we have shown to abrogate the cell surface expression of HLA-DQ. The observed reduction in IFN-γ-induced antigen expression in serum-supplemented medium was most probably a result of the greater degree of KC differentiation observed under these culture conditions.

The rarity of HLA-DQ expression on keratinocytes *in vivo* could well be because of the intracellular localization of this molecule which may not be detected by conventional staining techniques. It has been demonstrated that the cell surface expression of HLA-DR is five times greater than that of HLA-DQ,[29] and shedding of the HLA-DQ molecule has also been postulated as a reason for its limited cell surface occurrence.[31] Even in the inflammatory dermatoses, characterized by intense KC expression of HLA-DR, the concomitant appearance of HLA-DQ is unusual, and to date has only been observed in cutaneous T cell lymphoma, lichen planus,[14] *Borrelia*-induced skin disease,[15] and allergic and irritant contact dermatitis.[16] This may be a marker of different KC function in these diseases, but is more probably a result of higher concentrations of IFN-γ produced by dermal or intra-epidermal activated T lymphocytes in these particular dermatoses. An analogous situation has been observed in auto-immune thyroid disease where in class II antigen expression by thyrocytes HLA-DR is more commonly and intensely expressed than HLA-DQ.[32] The same pattern is seen in cultured thyrocytes treated with IFN-γ.[32] Measurement of steady-state mRNA levels transcribed from different HLA-DQ loci may allow further identification of the genes upon which IFN-γ acts in human KC. Further study of the regulation of HLA-DQ is required since one allele (HLA-DQβ) has recently been found to be associated with pemphigus vulgaris.[33] Furthermore, HLA-DQ expression by lymphoid cells has been associated with antigen-specific suppression of the immune response.[34] Since IFN-γ treated KCs have also been observed to induce antigen-specific unresponsiveness,[35] it will be of interest to determine whether such reactions are mediated via HLA-DQ.

The ability of IFN-γ to induce ICAM-1 expression on the surface of KC and the intense cell surface expression of this antigen is of major importance in the lymphocyte–KC adherence reaction *in vitro*[24,25] and may also be relevant in many inflammatory dermatoses *in vivo.*[20] It has been demonstrated that anti-LFA-1 and anti-ICAM-1 antibodies, but not anti-HLA-DR antibodies, inhibit the binding of T lymphocytes to IFN-γ-treated KC[24–26] thus producing additional evidence that ICAM-1 is involved in this process. The prominent cell-surface expression of this antigen by KC after IFN-γ exposure supports this idea.

Thus, it is postulated that activated T lymphocytes in the dermis induce ICAM-1 expression on KC by means of IFN-γ production. The present work extends our previous suggestion that IFN-γ is the common molecule responsible for the expression of HLA-DR on KC in a variety of inflammatory dermatoses.[36] ICAM-1 expression of KC could promote lymphocyte–KC adherence reactions and the subsequent events leading to class II antigen induction on KC, which may contibute to the pathophysiological changes observed in a variety of inflammatory dermatoses.[37]

ACKNOWLEDGMENTS

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REFERENCES

1 Giles RC, Capra JC. Structure, function and genetics of human class II molecules. In: *Advances in Immunology,* (Dixon FJ, ed.) London: Academic Press, 1985, 37: 1–71

2 Hammerling GJ. Tissue distribution of Ia antigens and their expression on lymphocyte sub-populations. *Transplant Rev* 1976, **30:** 64–82.

3 Natali PG, Segatto O, Ferrone S *et al.* Differential tissue distribution and ontogeny of DC-1 and HLA-DR antigens. *Immunogenetics* 1984; **19:** 109–16.

4 Rosenthal AS, Shevach EM. Function of macrophages in antigen recognition by guinea-pig T lymphocytes I. Requirement for histocompatible macrophages and lymphocytes. *J Exp Med* 1973, **138:** 1194–212

5 Weinberger O, Herrman SH, Mescher MF *et al.* Cellular interactions in the generation of cytotoxic T-lymphocyte responses: role of Ia positive splenic adherent cells in presentation of H-2 antigen. *Proc Natl Acad Sci USA* 1980; **77:** 6091–95.

6 Raff HV, Picker LJ, Stobo J. Macrophage heterogeneity in man. a sub-population of HLA-DR bearing macrophages required for antigen induced T cell activation also contains stimulators for autologous-reactive T cells. *J Exp Med* 1980; **152:** 581–93

7 Gonwa TA, Picker LJ, Raff HV *et al.* Antigen-presenting capabilites of human monocytes correlates with their expression of HLA-DS, and Ia determinant distinct from HLA-DR. *J Immunol* 1983; **130:** 706–11.

8 Natali PG, de Martino C, Quaranta V *et al.* Expression of Ia-like antigens in normal non-lymphoid tissues. *Transplantation* 1981; **31:** 75–8.

9 Stingl G, Tamaki K, Katz SI. Origin and function of epidermal Langerhans cells. *Immunol Rev.* 1980; **53;** 149–74.

10 Murphy GF, Shepard RS, Harrist TJ *et al.* Ultrastructural documentation of HLA-DR antigen reactivity in normal human acrosyringial epithelium. *J Invest Dermatol* 1983; **81:** 181–3.

11 Messadi DV, Pober JS, Murphy GF. Effects of recombinant-interferon-γ on HLA-DR and HLA-DQ expression by skin cells in short-term organ culture. *Lab Invest* 1988; **58:** 61–7.

12 Aubock H, Romani N, Grubauer G, Fritsch PI. HLA-DR expression on keratinocytes is a common feature of diseased skin. *Br J Dermatol* 1986; **114:** 465–72.

13 Shiohara T, Moriya N, Tanaka Y *et al.* Immunopathologic study of lichenoid skin diseases: Correlation between HLA-DR positive keratinocytes or Langerhans cells and epidermotropic T cells. *J Am Acad Dermatol* 1987; **18:** 67–74.

14 Volc-Platzer B, Groh V, Wolff K. Differential expression of class II alloantigens by keratinocytes in disease. *J Invest Dermatol* 1987; **89:** 64–8.

15 Tjernlund U, Scheynius A, Asbrink E, Hovmark A. Expression of HLA-DQ antigens on keratinocytes in *Borrelia* spirochete-induced skin lesions. *J Immunol* 1986; **23:** 383–8.

16 Gawkrodger DJ, Carr MM, McVittie E *et al.* Keratinocyte expression of MHC class II antigens in allergic sensitization and challenge reactions and in irritant contact dermatitis. *J Invest Dermatol* 1987; **88:** 11–16.

17 Basham TY, Nickoloff BJ, Merigan TC, Morhenn VB. Recombinant gamma interferon induces HLA-DR expression on cultured human keratinocytes. *J Invest Dermatol* 1984; **83:** 88–90.

18 Nathan CF, Kaplan G, Lewis WR *et al.* Local and systemic effects of intradermal recombinant interferon-gamma in patients with systemic leprosy. *N Engl J Med* 1986; **315:** 6–11.

19 Basham TY, Nickoloff BJ, Merigan TC, Morhenn VB. Recombinant gamma interferon differentially regulates class II antigen expression and biosynthesis on cultured normal human keratinocytes. *J Interferon Res* 1985; **5:** 23–7.

20 Griffiths CEM, Voorhees JJ, Nickoloff BJ. Characterization of intercellular adhesion molecule-1 and HLA-DR expression in normal and inflamed skin: modulation by recombinant interferon-γ and tumor necrosis factor. *J Am Acad Dermatol* (in press).

21 Dougherty GJ, Murdoch S, Hogg N. The function of human intercellular adhesion molecule-1 (ICAM-1) in the generation of an immune response. *Eur J Immunol* 1988; **18:** 35–9.

22 Dustin ML, Rothlein R, Bhan AK *et al.* Induction by IL-1 and interferon-γ: Tissue distribution, biochemistry, and function of a natural adherence molecule (ICAM-1). *J Immunol* 1986; **137:** 245–54.

23 Marlin SD, Springer TA. Purified intercellular adhesion molecule-1 (ICAM-1) is a ligand for lymphocyte function-associated antigen-1 (LFA-1). *Cell* 1987; **51:** 813–19.

24 Nickoloff BJ, Lewinsohn D, Butcher EC *et al.* Recombinant gamma interferon increases the binding of peripheral blood mononuclear leukocytes and a leu-3^{+} T lymphocyte clone to cultured keratinocytes and to a malignant cutaneous squamous cell carcinoma line that is blocked by antibody against the LFA-1 molecule. *J Invest Dermatol* 1988; **90:** 17–22.

25 Nickoloff BJ, Mitra RS. Phorbol treatment enhances binding of mononuclear leukocytes to autologous and allogeneic gamma interferon treated keratinocytes which is blocked by anti-LFA-1 monoclonal antibody. *J Invest Dermatol* 1988; **90:** 684–9.

26 Dustin ML, Singer KH, Tuck DT, Springer TA. Adhesion of T lymphoblasts to epidermal keratinocytes is regulated by interferon-γ and is mediated by intercellular adhesion molecule-1 (ICAM-1). *J Exp Med* 1988; **167:** 1323–40.

27 Liu SC, Karasek M. Isolation and growth of adult human epidermal keratinocytes in cell culture. *J Invest Dermatol* 1978; **71:** 157–62.

28 Boyce ST, Ham RG. Calcium regulated differentiation of normal human epidermal keratinocytes in chemically defined clonal culture and serum-free serial culture. *J Invest Dermatol* 1983; **81** (Suppl.): 33–40.

29 Chen Y-X, Evans RL, Pollack MS *et al.* Characterization and expression of the HLA-DC antigens defined by anti-Leu 10. *Hum Immunol* 1984; **10:** 221–35.

30 Wikner NE, Huff JC, Norris DA *et al.* Study of HLA-DR in cultured human keratinocytes. *J Invest Dermatol* 1986; **87:** 559–64.

31 Capobinanchi MR, Ameglio F, Tosi R, Dolei A. Differences in the expression of HLA-DR, BR and DQ molecules in human cells treated with recombinant interferon gamma: comparison to other interferons. *Hum Immunol* 1985; **13:** 11.

32 Todd I, Pujol-Borrell R, Abdul-Karim AS *et al.* HLA-D Subregion expression by thyroid epithelium in autoimmune thyroid diseases and induced *in vitro*. *Clin Exp Immunol* 1987; **69**: 532–42.

33 Sinha AA, Brautbar C, Szafer F *et al.* A newly characterized HLA-DQ allele associated with pemphigus vulgaris. *Science* 1988; **239**: 1026–9.

34 Hirayama K, Matsusuita S, Kikuschi I *et al.* HLA-DQ is epistatic to HLA-DR in controlling the immune response to schistosomal antigen in humans. *Nature* 1987; **327**: 426–30.

35 Gaspari A, Jenkins M, Katz SI Induction of antigen-specific T cell unresponsiveness by Ia$^+$ keratinocytes. *J Invest Dermatol* 1988; **90**: 562.

36 Nickoloff BJ, Basham TY, Merigan TC, Morhenn VB. Keratinocyte class II histocompatibility antigen expression. *Br J Dermatol* 1985; **112**: 373–4.

37 Nickoloff BJ. The role of gamma interferon in cutaneous trafficking of lymphocytes with emphasis on molecular and cellular adhesion events. *Arch Dermatol* (in press).

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Keratin gene expression during the resolution of psoriatic plaques: effect of dithranol, PUVA, etretinate and hydroxyurea regimens

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SUMMARY

Quantitative changes in the levels of keratin polypeptides extracted from keratotome shavings from psoriatic epidermis were measured by using one-dimensional SDS-PAGE, followed by scanning densitometry. Values obtained were compared with results for non-lesional epidermis and from epidermis from normal individuals. Patients on four different treatment regimens were investigated by repeated sampling over 3–4 months starting before therapy commenced. The levels of four keratins changed significantly: keratins 1 (70 kd) and 2 (66 kd) tended to rise to normal levels, while keratins 16 (50 kd) and 18 (44 kd) fell to normal levels. There were differential effects as well as differences in the rates of normalization depending upon the treatment regimen. The most rapid normalization of the levels of all four keratins was observed with topical dithranol (anthralin) treatment (five patients) with plaque resolution and keratin level normalization after 7–9 weeks. Oral hydroxyurea (three patients) had similar effects, but over a longer time scale (20 weeks). In contrast, oral etretinate (four patients) caused a normalization of all except keratin 2 (66 kd) over a period of 20–28 weeks, and keratin 1 (70 kd) levels tended to 'overshoot' the normal level. PUVA (five patients) caused rapid normalization (in 9–12 weeks) of keratins 2 (66 kd) and 18 (44 kd), but had much weaker effects on keratins 1 (70 kd) and 10 (57 kd). These results suggest that resolution of lesions as judged by clinical criteria can occur without normalization of the keratin electrophoretic profile. Possibly the most reliable marker of clinical resolution was the reduction in keratin 16 (50 kd), since treatment effects on the differentiation of keratins 1 (70 kd) and 2 (66 kd) were different.

The epidermis comprises two compartments: the undifferentiated proliferative cells of the basal layer, and the differentiating cells of the suprabasal layers. Of the approximately 20 cytokeratins

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described in human epithelia,[1] not only are different cytokeratins detected in different tissues, but also different cells express only a few of these polypeptides, usually as co-ordinate pairs. In human epidermis, the proliferating basal layer cells synthesize keratins 5 and 14 (59 kd and 52 kd) and the differentiating cells synthesize keratins 1 and 10 (70 kd and 57 kd).[2] These latter are used as markers of differentiation.

In psoriasis, epidermal keratinocytes fail to undergo, or fail to complete, normal terminal differentiation, possibly as a consequence of reduced cell turnover time which some workers believe to be less than 5 days compared with the normal 28 days. In both normal and psoriatic epidermis there is an equilibrium between cell formation through division of cells of the basal layer and cell loss by desquamation of the dead cells of the stratum corneum, although the mechanism whereby this equilibrium is maintained is unknown.[3] Analysis of the epidermal keratins by electrophoretic methods reveals that a modified set of keratins is expressed in psoriasis, and that successful treatment (i.e. resolution of a plaque) restores the normal keratin set.[4,5] The most frequently reported changes in the keratins expressed by psoriatic epidermis are a reduction in the amount of neutral-to-basic high molecular weight keratin 1[6] and an increase in the amounts of, or appearance of, polypeptides of molecular weight 56,000 and 50,000/48,000.[7,8]

Previous studies of changes in keratin polypeptide patterns during the resolution of a psoriatic plaque have been performed over short treatment periods with only semi-quantitative analyses of the amounts of the polypeptides. We decided to initiate a longer term study in which the keratin polypeptides were analysed as far as possible under rigorously standardized conditions, the keratin polypeptides being quantified with a laser scanning densitometer following gel electrophoresis. Epidermal samples were taken at frequent intervals until clinical resolution. Patients received one of four different therapeutic regimens: dithranol (anthralin), PUVA, etretinate or hydroxyurea. We were interested in examining the relationship between the keratin polypeptide profiles and the stage of activity of psoriatic lesions and in particular to find reproducible parameters that would indicate clinical efficacy.

METHODS

Subjects

Seventeen patients with generalized plaque psoriasis, 10 male and seven female undergoing four different treatment regimens were sampled repeatedly, with their informed consent, according to their availability and willingness to donate samples of epidermis. All patients except one had received no treatment for at least 2 months prior to sampling. The remaining patient changed treatments at the start of the study owing to unresponsiveness to previous therapies.

The four treatment regimens were dithranol (anthralin): dithranol at increasing strength, up to 2%, with daily tar baths or UVB or both dependent on patient reaction (five patients); PUVA: 0·6 mg/kg body weight 8-methoxypsoralen orally together with a mean dose of 3·0 J/cm^2 of UVA radiation, three times per week (five patients); etretinate treatment: Tigason® at an oral dose of 0·75 mg/kg body weight daily (four patients), and hydroxyurea treatment: hydroxyurea at a mean oral dose of 1000 mg daily (three patients). Clinicians tend to use etretinate or hydroxyurea only if dithranol or PUVA treatments have been unsuccessful, depending upon patient tolerance, limitations on PUVA dosage and other factors. Only patients whose psoriasis underwent clinical resolution during treatment are reported here. Normal skin samples were obtained for comparison from subjects attending a local hair transplant clinic and from students and laboratory personnel, none of whom had a history of skin disorders.

Epidermis

Keratotome shavings were taken with a Castroviejo electrokeratotome (Storz Instrument Co.) using a standard shim setting of 0·1 mm, from normal epidermis and from lesional and uninvolved epidermis from the psoriatic patients. In all cases the site was either on the arm or leg. Although psoriatic plaque epidermis tends to be thickened, the standard shim setting was maintained, care being taken to try to obtain samples of approximately the same thickness. This was achieved by first removing superficial scale by three successive applications of 5 × 2 cm strips of adhesive tape (Blenderm surgical tape, 3M). Shavings were taken prior to treatment and at intervals during treatment, from different areas of the same lesion. Only large lesions were sampled, in order to avoid any effect of wound healing on the resolution pattern. Lesions were photographed at the time of sampling.

Extraction of keratins

Keratins were extracted by a modification of the procedure of Skerrow.[9] Briefly, epidermal samples were homogenized and then sonicated in 0·1 M citric acid/sodium citrate buffer, pH 2·65, at 4°C, followed by centrifugation at 50,000 ***g*** for 30 min. The keratins in the supernatant were partially purified by serial isoelectric precipitation at pH 4·0. Stratum corneum keratins were extracted by incubating the strips of adhesive tape in 0·05 M Tris/HCl buffer pH 6·8 containing 1% (w/v) SDS, 1% (v/v) 2-mercaptoethanol, 0·005% (w/v) bromophenol blue and 20% (v/v) glycerol, at 40°C for 60 min.

One-dimensional electrophoresis

Samples extracted with citric acid/sodium citrate buffer were adjusted to pH 7·0 and boiled for 2 min with SDS (1%) and 2-mercaptoethanol (2%). Glycerol (20%) and bromophenol blue (0·005%) were then added and the keratin samples were analysed by SDS/polyacrylamide slab gel electrophoresis on a 7·5–17·5% gradient (acrylamide/bisacrylamide ratio 75:2) with a 4% (w/v) stacking gel, in a discontinuous Tris/glycine buffer system.[10] In each run, 6 μg phosphorylase *a* (M_r 94,000) was added to the keratin sample prior to analysis to act as an internal standard. Gels were fixed overnight and stained with 0·1% Coomassie brilliant blue R.

Two-dimensional electrophoresis

Two-dimensional electrophoretic analysis was carried out by a combination of isoelectric focusing (IEF) in the first dimension on rod gels and in the second dimension by SDS/PAGE as described above. The procedures used were a combination of two methods.[11,12] Concentrated keratin samples (5–10 mg/ml) were boiled in 1% SDS and 1% 2-mercaptoethanol, and then adjusted to 9·5 M in urea, 2% (v/v) in Nonidet P-40 and 5% (v/v) in 2-mercaptoethanol. Aliquots containing 100 μg protein were loaded onto 4·0% polyacrylamide tube gels containing 2·0% (v/v) Nonidet P-40, 9·5 M urea and 2% (v/v) Ampholines (LKB, a mixture of pH 5–8 and pH 3·5–5 ranges, ratio 6:1). Electrophoresis was performed for 20 h at 400V and for 2 h at 800V. The gels were then equilibrated in 0·05 M Tris/HCl pH 6·8 containing 1% SDS, 1 mM EDTA and 0·5% 2-mercaptoethanol for 30 min, followed by electrophoresis in the second dimension.

Immunoblotting

Two-dimensional gels were immunoblotted using the method of Towbin *et al.*[13] The blots were labelled with monoclonal antibodies AE1 and AE3 (gifts from Dr T.T.Sun, The Johns Hopkins School of Medicine, Baltimore, MD 21205, U.S.A.) and visualized by staining with rabbit anti-mouse IgG followed by *Staph. aureus* protein A labelled with ^{125}I, and autoradiography.

Scanning densitometry

Stained, wet one-dimensional gels were scanned using an LKB Ultroscan Laser Densitometer. Absolute amounts of keratins in a mixture, rather than relative amounts were calculated. The amount of each keratin was normalized to the standard and corrected for molecular weight in relation to Coomassie blue binding to give a molar ratio value of keratin : phosphorylase *a*.[14] To facilitate a quantitative comparison for each keratin polypeptide in different samples from one track or gel to another, the sum of the normalized molar ratios (keratin : phosphorylase *a*,) per track was adjusted to give values as if exactly 20 μg total keratin polypeptides had been applied to the gel. The normalized molar ratios of individual keratin polypeptides were then altered proportionately. It was found that the normalized molar ratios of keratin : phosphorylase *a* of the same sample across one gel or on different gels agreed well (reproducibility better than ± 5%). Results were analysed using Student's unpaired *t*-test.

Changes in amounts of keratin polypeptides during therapy were calculated by comparison with pretreatment levels. For each individual keratin polypeptide, changes in levels were plotted against time and the best fitting line through the individual values for each treatment group was calculated using a computer fit to $y = k_1 \ (1 - e^{-k_2 x})$.

RESULTS

Keratins from living epidermal cells

Keratins were characterized on the basis of their molecular weight, pI value and immunoreactivity and the nomenclature of Moll *et al.*[1] was adopted. The higher molecular weight polypeptides, keratins, 1, 2, 5, and 6 (70 kd, 66 kd, 59 kd and 56 kd) had relatively basic isoelectric variants in the region pH 6·8–7·9, the smaller keratins 10, 14, and 16 (57 kd, 52 kd and 50 kd) had variants in the acidic region (pH 5·0–5·3), while keratins 7, 8, 17, and 18 (54 kd, 52′ kd, 46 kd and 44 kd) had variants in the region pH 5·6–6·0. Immunoblotting showed that all acidic keratins were AE1 positive. The AE1 antibody stained keratins 10 and 14 less intensely than keratin 16 in both one- and two-dimensional gel electrophoresis. The AE3 antibody stained the basic to neutral keratins.[1,2,5,7,8,18]

In one-dimensional electrophoresis (Fig. 1), a major difference was that, compared with normal epidermis, the levels of keratin 1 (70 kd) in uninvolved and lesional (untreated) psoriatic epidermis were reduced. In lesional, but not in uninvolved epidermis, there was also the virtual disappearance of keratin 2 (66 kd) with heavier representation of keratins 16 (50 kd) and 18 (44 kd). In addition, two dimensional electrophoresis showed the presence in involved psoriatic epidermis of keratins 6 (56 kd) and 8 (52′ kd).

These observations were confirmed by comparison of scanning densitometry data for keratins obtained from normal epidermis (10 subjects), uninvolved psoriatic epidermis (five patients), lesional psoriatic epidermis and resolved lesional epidermis (a total of 14 patients from four different treatment groups) from the same anatomical sites, i.e. arm and leg. Statistical analysis (unpaired *t*-test) showed that normal and resolved epidermis were closely similar in polypeptide composition and that resolved and uninvolved epidermis did not differ significantly, whereas in uninvolved epidermis keratin 1 was significantly lower than normal ($P < 0{\cdot}05$). In lesional epidermis keratins 1 and 2 were both significantly lower ($P < 0{\cdot}0001$) while keratins 16 and 18 were elevated to significantly higher levels ($P < 0{\cdot}0001$) compared with resolved, uninvolved and normal epidermis (Fig. 2). The remainder of the polypeptides showed the following differences: keratin 14 was present at higher levels in lesional epidermis than in uninvolved and normal epidermis ($P < 0{\cdot}05$). Keratin 17 was found at higher levels in lesional epidermis than in

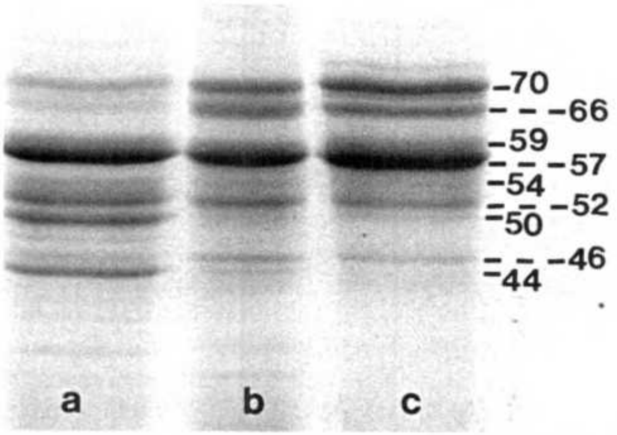

FIGURE 1. SDS-PAGE on 7·5–17·5% gradient gel with keratins extracted from living cells of (a) psoriatic lesion (leg), (b) non-lesional epidermis from the same psoriatic individual (leg), and (c) normal epidermis from a disease-free individual (leg). Numbers are molecular weights in kd.

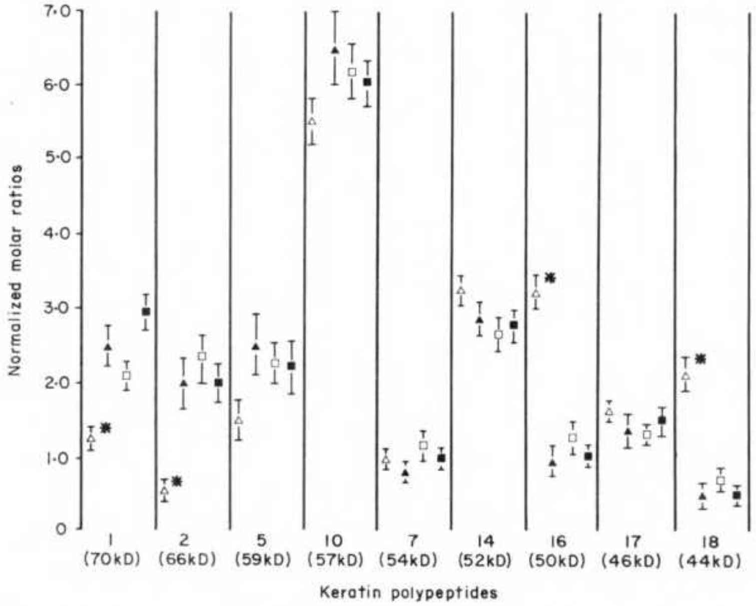

FIGURE 2. Comparison of amounts of keratin polypeptides based on scanning densitometry of SDS-PAGE. All samples were from sites on either the arm or leg. (△) Psoriatic lesion, (▲) resolved lesion, (□) uninvolved epidermis, (■) normal epidermis from healthy individuals. The keratin polypeptides are identified by molecular weight in SDS-PAGE. The normalized molar ratios are given as means ± SEM. *Polypeptides that differ most significantly ($P < 0\cdot0001$), namely those for keratins 1, 2, 16 and 18, in lesional epidermis.

uninvolved epidermis ($P < 0{\cdot}05$), and in lesional epidermis keratins 5 and 10 were present at lower levels than in resolved epidermis ($P < 0{\cdot}05$).

Changes in keratins during resolution of psoriatic lesions with therapy

In all patients, we did not observe any differences in the rates of resolution of keratotomed lesions as compared with non-keratotomed lesions elsewhere on the same patient. It is also pertinent to mention that keratotomed lesions in patients unresponsive to treatment and not included in the present study, were not observed to resolve, suggesting further that wound healing was not affecting the resolution pattern.

The amounts of keratin polypeptides from living cells altered as the psoriatic lesion improved clinically (Fig. 3, a–f). The mean levels of each keratin from unresolved and resolved psoriatic lesions for each treatment group are given in Figure 4.

The initial low level of keratin 1 increased to levels comparable with those in normal epidermis at 6 and 18 weeks during lesion resolution with dithranol and hydroxyurea therapies, respectively. This normalization immediately preceded clinical resolution which was not considered complete until 7–9 and 20 weeks, respectively. The low level of keratin 1 remained unchanged during PUVA treatment although lesion resolution in clinical terms had taken place at 9–12 weeks. An apparent 'over-production' of this keratin occurred during etretinate therapy. Levels of this polypeptide were 'normal' by 12 weeks of treatment but clinical resolution was not complete until a later stage i.e. 20–28 weeks.

The amounts of keratin 2 increased during dithranol, PUVA and hydroxyurea treatments to levels greater than those in normal epidermis at 4, 9 and 14 weeks, respectively. These changes were earlier than for keratin 1 and preceded clinical resolution. In contrast, only minor increases in levels of this keratin were observed during etretinate therapy for up to 28 weeks.

During lesion resolution with dithranol and hydroxyurea treatments, keratin 10 increased to normal and greater than normal levels respectively within 3 weeks of therapy. The contrasting effects of PUVA and etretinate therapies on changes in levels of keratin 1 were mirrored in the changes in keratin 10 level which greatly increased during lesion resolution on etretinate therapy whereas PUVA treatment had a negligible effect. Keratins 1 and 10 are believed to be expressed as a pair in differentiating keratinocytes. The changes in levels of these two keratins corresponded, i.e. there was no increase in keratins 1 and 10 during PUVA therapy and large increases in both keratins during etretinate therapy. However, these changes were not coincident; increases in levels of keratin 10 preceded those of keratin 1.

Keratins 5 and 14 are normally expressed as a pair in the proliferating basal cells of the epidermis. The levels of keratin 14 were found to decrease during etretinate and hydroxyurea therapies. These changes seemed at variance with those of keratin 5 levels which remained unchanged in lesions resolved with etretinate, but increased during dithranol, hydroxyurea and PUVA therapies. However, these changes brought the keratin pairs to matching levels i.e. normality or below normality, in lesions resolved with dithranol, etretinate or hydroxyurea although these pairs remained unco-ordinated after PUVA therapy.

Pre-treatment levels of keratin 16 were found to be higher in those patients selected for etretinate and hydroxyurea therapies than in those on other therapies. However, during clinical resolution with all four treatment regimens, the levels of keratin 16 decreased and normalized. The rates of these changes were slower than for keratins 2, 10, 5 and 18, but similar to keratin 1, with normal levels being reached 1 to 2 weeks prior to clinical resolution.

Etretinate produced the slowest change in keratin 18. About 25 weeks were required for it to stabilize and even then the mean levels had not normalized coincident with the clinical

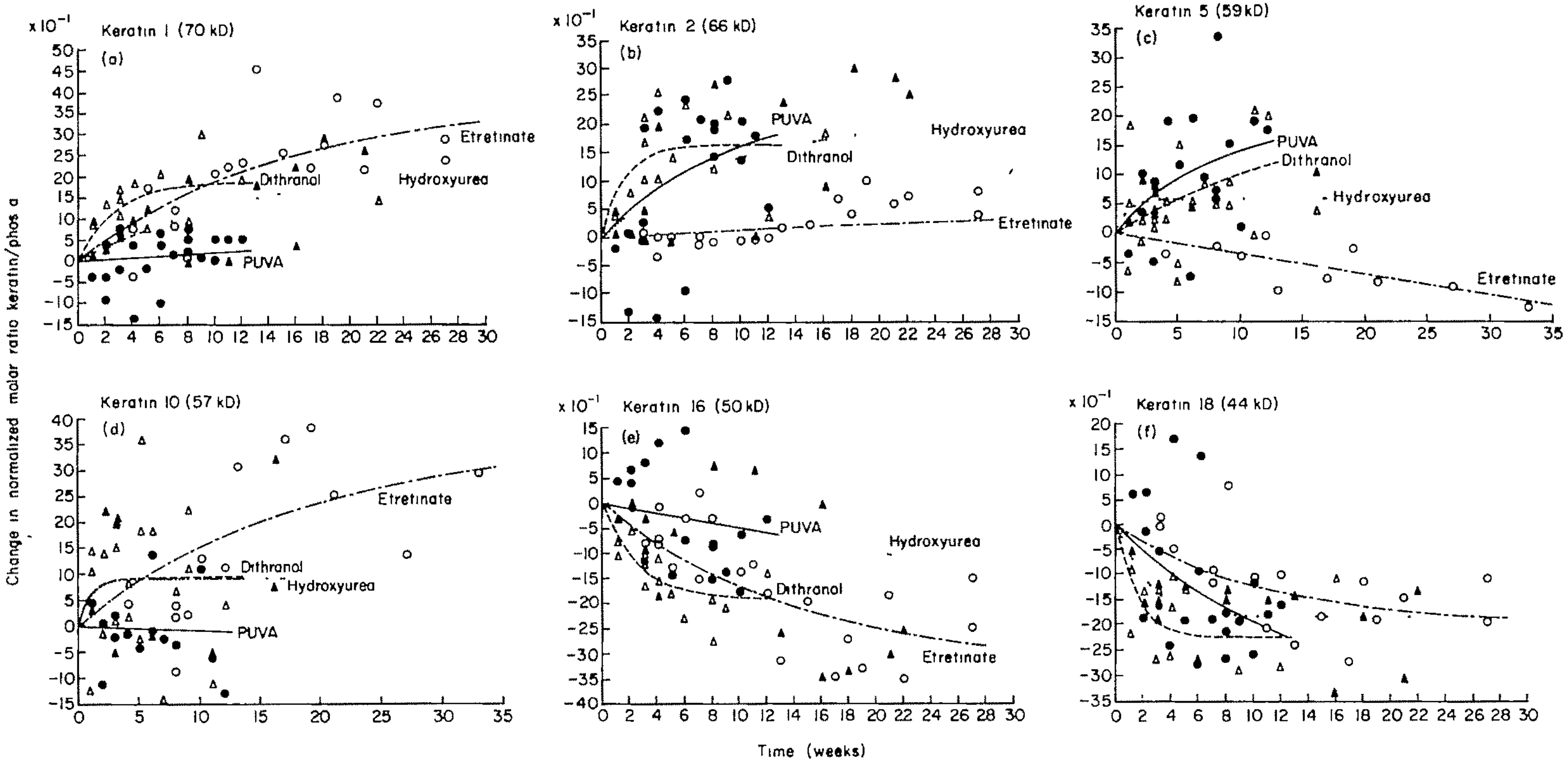

FIGURE 3. Changes in molar ratios of keratins during plaque resolution with different treatment regimens: (a) keratin 1 (70 kd), (b) keratin 2 (66 kd), (c) keratin 5 (59 kd), (d) keratin 10 (57 kd), (e) keratin 16 (50 kd), and (f) keratin 18 (44 kd). (– –△– –) dithranol (five patients), (▲ · ·) hydroxyurea (3), (— ○ —) etretinate (4), (—●—) PUVA (5). The lines were generated from a computer fit of the sets of data to $y = k_1 (1 - e^{-k_2 x})$

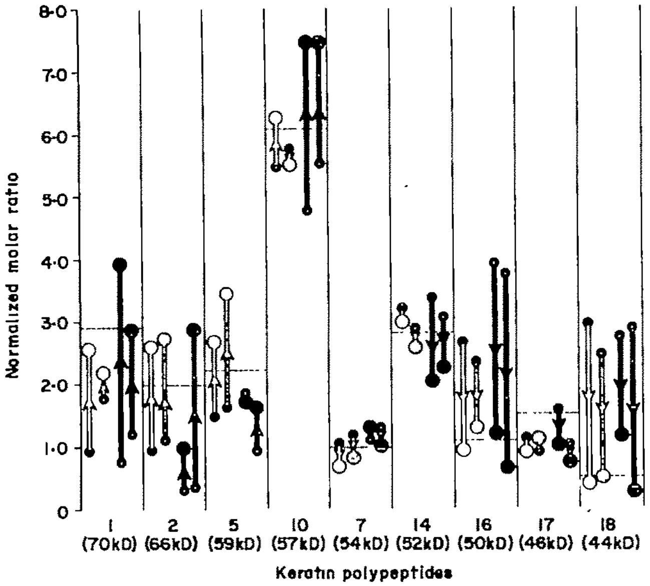

FIGURE 4. Effect of four treatment regimens on the mean levels of keratin polypeptides expressed as normalized molar ratios. Values for pre-treatment and resolved lesional epidermis are shown and the direction of the change in level of a particular keratin is shown by the arrow: (○) dithranol, (○) PUVA (●) etretinate, (●) hydroxyurea. Dashed lines indicate keratin levels in normal epidermis

resolution. Normal levels of this keratin were achieved quite rapidly with the other therapies i.e. after 3 weeks of dithranol, 8 weeks of PUVA and 6 weeks of hydroxyurea.

For the remaining keratins found in psoriatic lesions the situation can be summarized as follows: keratins 7 and 17 remained either unchanged, or were decreased by insignificant amounts during lesion healing. Keratins 6 and 8 were only resolved by two-dimensional electrophoresis. This made it impossible to observe any quantitative relationship between their disappearance and clinical resolution.

Keratins from stratum corneum

Keratins from superficial scales of untreated psoriatic lesions had electrophoretic profiles similar to those of keratins from the living cells. These profiles were abnormal when compared with uninvolved psoriatic and normal stratum corneum which contained only three major keratins with molecular weights 65 kd, 57·5 kd and 55 kd. During lesion resolution, stratum corneum keratin profiles returned to those of normal or uninvolved epidermis with the disappearance of 70 kd, 66 kd and lower molecular weight polypeptides. Normalization of the stratum corneum keratin profile occurred at the same time as a large decrease in keratin 16 in the living cells, regardless of treatment.

DISCUSSION

Involved psoriatic epidermis contained extra polypeptides of 56 kd (AE3-positive pI 7·9, gene family II, no. 6) and 50 kd (AE1-positive, pI 5·1, gene family I, no. 16) confirming the findings of Weiss *et al.*[8] and of McGuire *et al.*[7] Keratin 16 reacted most strongly with monoclonal antibody AE1 and probably corresponds with the 48 kd keratin of Weiss *et al.*[8] We observed the presence of additional keratins of 52′ kd (no. 8) and 44 kd (no. 18) in psoriatic epidermis, but on the basis of the present data it is uncertain whether these are native keratins or are partial degradation products of high molecular weight keratins. Normally, keratin 18 is reported to be present in simple epithelia or in Merkel cells.

In addition, in involved psoriatic epidermis the amounts of keratins 1 and 2 were low, as has been reported previously by several groups. It was also noted that levels of keratin 1 were significantly reduced in uninvolved psoriatic epidermis, as reported previously, although resolved epidermis (theoretically the same as uninvolved epidermis) was within the range of normality. These low levels could result from diminished synthesis in psoriasis or more rapid degradation; greater proteolytic activity has been observed in psoriatic epidermis.[15] However, it is also important to bear in mind that the spectrum and levels of keratins expressed will reflect the relative numbers of the different cell types present in a particular sample of epidermis. In psoriatic plaque the proportion of viable differentiated cells relative to the number of germinative cells is lower (a 1:1 ratio) than in normal epidermis where there is a 2:1 ratio.[3]

Certainly, the keratin profiles in psoriasis are complex, with the presence of differentiation markers (keratins 1, 2, and 10), basal cell keratins (14 and 5) as well as hyperproliferative markers (keratins 16 and 6). From our investigation it is clear that the spectrum of keratin expression alters according to the clinical state of the lesion. Although keratin changes from lesions of non-responding patients are not included in this report, it was observed that the clinical deterioration of such patient's psoriasis was accompanied by keratin changes which were practically the reverse of those observed during resolution in responsive patients. Occasionally, early in treatment when it is not obvious clinically whether resolution is beginning to occur, the changes in keratin patterns may provide useful confirmation. In general, resolution of a lesion involved an increase in the amounts of high molecular weight keratins (predominantly gene family II) and a reduction in the levels of some of the low molecular weight keratins (predominantly gene family I). However, different types of changes in the keratin patterns as well as differences in the time scale of normalization of the keratins and clinical resolution were observed depending upon the treatment regimen. These differences possibly reflect the different routes of administration and modes of action of the therapeutic agents. It has been reported that 6 h after dithranol treatment was started the cell cycle time appeared to be reduced from 132 h to 56 h which may explain its relatively rapid effect in psoriasis.[16] Recently, Tucker *et al.*[17] showed that dithranol inhibited the effects of calmodulin which is believed to have a role in DNA repair. Hydroxyurea is an antimitotic agent, inhibiting DNA synthesis by blocking the conversion of ribonucleotides to deoxyribonucleotides. It will have its major effect in inhibiting division of the cells of the germinative compartment. The action of PUVA as a cytotoxic agent on the basal cells is contentious while the modes of action of retinoids are not yet clear.

Topical dithranol and oral hydroxyurea produced similar changes, the most significant in keratins 1, 2, 16 and 18. Etretinate therapy produced striking increases in the differentiation marker keratins 1 and 10, although no change in keratin 2, but simultaneously caused a decrease in the levels of the hyperproliferation marker keratin 16. Such a significant increase in levels of differentiation markers was unexpected from a retinoid. The changes in the keratins occurred relatively slowly (20–25 weeks). In contrast, Staquet *et al.*[4] reported normalization of the stratum corneum keratin pattern of lesional epidermis after only 4 weeks of etretinate therapy, although the changes in amounts of keratins they observed were rather small. In PUVA treatment, the levels of the differentiation markers (keratins 1 and 10) did not increase significantly, remaining lower than normal, although there was a significant increase in levels of keratin 2 and a decrease in amounts of keratin 16 with lesion resolution. Consequently, it would appear that the reduction in the level of keratin 16 was the most general and reliable marker of clinical resolution in psoriasis.

It is necessary to explain why some psoriatic lesions appear to undergo clinical resolution and yet still have 'abnormal' levels of keratins 1 and 2. It has been reported on the basis of peptide mapping that keratins 1 and 2 are very closely related, and, according to Fuchs and Green,[18] the synthesis of keratin 1 precedes that of keratin 2 by several epidermal layers. This would seem to agree with Staquet *et al.*[4] who recently reported that keratin 2 (65 kd) was a major component of granular cells. Possibly an excess of keratin 1 in some way compensates for the lack of keratin 2 or *vice versa*.

The increase in differentiation markers represents a return to normal cell maturation, suggesting that the 'surviving' basal cells express their characteristic keratins with a resumption of normal keratinization. The fact that the levels of keratin 14 remained relatively unchanged throughout lesion resolution may indicate that some basal cells are 'non-hyperproliferative'. Selective effects of cytotoxic drugs on hyperproliferative cells are thought to be a consequence of their action on the different functional DNA synthesis pathways of these cells.[19] The concept of two or more kinetically distinct subpopulations in the growth fraction of psoriatic plaque was suggested by Potten.[20]

Although there is controversy concerning the cell kinetics in psoriasis,[21] it is well established that there is increased cell proliferation. However, it is not clear whether the failure of maturation of keratinocytes is a consequence or a cause of this phenomenon. Fry and McMinn[22] found that in the resolution of psoriasis the granular layer showed signs of reformation before there was a fall in the mitotic activity of the epidermis. The finding in the present study of earlier changes in keratins 2 and 10, both polypeptides of differentiating cells and in particular granular cells, preceding a fall in level of the proliferation marker keratin 16, may lend support to this observation.

REFERENCES

1 Moll R, Franke WW, Schiller DL. The catalog of human cytokeratins: patterns of expression in normal epithelia, tumours and cultured cells. *Cell* 1982; **31:** 11–24.

2 Eichner R, Bonitz P, Sun TT. Classification of epidermal keratins according to their immunoreactivity, isoelectric point, and mode of expression. *J Cell Biol* 1984; **98:** 1388–1396.

3 Heenan M, Galand P. The kinetic alteration in psoriasis. In: *Psoriasis: Cell Proliferation* (Wright NA, Camplejohn RS, eds). Edinburgh: Churchill Livingstone, 1983; 218–26.

4 Staquet MJ, Faure MR, Reano A *et al.* Keratin polypeptide profile in psoriatic epidermis normalised by treatment with etretinate (aromatic retinoid Ro 10-9359). *Arch Dermatol Res* 1983; **275:** 124–9.

5 Levine MJ, McGilvray N, Baden HP. Effect of therapy on keratin polypeptide profiles of psoriatic epidermis. *Arch Dermatol* 1980; **116:** 1028–30.

6 Hunter I, Skerrow D. The proteins of living psoriatic epidermis. *Biochim Biophys Acta* 1981; **714:** 164–9.

7 McGuire J, Osber M, Lightfoot L. Two keratins MW 50,000 and 56,000 are synthesized by psoriatic epidermis. *Br J Dermatol* 1984; **111:** 27–37.

8 Weiss RA, Eichner R, Sun TT. Monoclonal antibody analysis of keratin expression in epidermal diseases: a 48- and 56-k dalton keratin as molecular markers of hyperproliferative keratinocytes. *J Cell Biol* 1984; **98:** 1397–406.

9 Skerrow D. The isolation and preliminary characterisation of human prekeratin. *Biochim Biophys Acta* 1977; **494:** 447–51.

10 Bowden PE, Cunliffe WJ. Modification of human prekeratin during epidermal differentiation. *Biochem J* 1981; **199:** 145–54.

11 O'Farrell PH. High resolution two dimensional electrophoresis of proteins. *J Biol Chem* 1975; **250:** 4007–21.

12 Ames GFL, Nikaido K. Two-dimensional gel electrophoresis of membrane proteins. *Biochemistry* 1976; **15:** 616–23.

13 Towbin H, Staehelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets. *Proc Natl Acad Sci USA* 1979; **76:** 4350–4.

14 Gilfix BM, Eckert RL. Coordinate control by vitamin A of keratin gene expression in human keratinocytes. *J Biol Chem* 1985; **260:** 14026–9.

15 Dubertret L, Bertaux B, Fosse M, Touraine R. Psoriasis: a defect in the regulation of epidermal proteases as shown by serial biopsies after cantharidin application. *Br J Dermatol* 1984; **110:** 405–10.

16 Steigleder GK, Pullman H. Control of antipsoriatic therapy by means of autoradiographic methods. In: *Psoriasis: Cell Proliferation* (Wright NA, Camplejohn RS, eds). Edinburgh: Churchill Livingstone, 1983; 317–26.

17 Tucker WGF, MacNeil S, Dawson RA *et al.* An investigation of the ability of antipsoriatic drugs to inhibit calmodulin activity: a possible mode of action of dithranol (anthralin). *J Invest Dermatol* 1986; **87:** 232–235.

18 Fuchs E, Green H. Changes in keratin gene expression during terminal differentiation of the keratinocyte. *Cell* 1980 **19:** 1033–42.

19 Newburger AE, Weinstein GD, McCullough JL. Biological and biochemical actions of methotrexate in psoriasis. *J Invest Dermatol* 1978; **70:** 183–6.

20 Potten CS, Wichmann HE, Loffler M *et al.* Evidence for discrete cell kinetic subpopulations in mouse epidermis based on mathematical analysis. *Cell Tiss Kin* 1982; **15:** 305–29.

21 Weinstein GD, Ross P, McCullough JL, Colton A. Proliferative defects in psoriasis. In: *Psoriasis: Cell Proliferation* Wright NA, Camplejohn RS, eds). Edinburgh, Churchill Livingstone, 1983, 189–208.

22 Fry L, McMinn RMH. Action of methotrexate on skin and intestinal epithelium in psoriasis. *Arch Dermatol* 1966; **93:** 726–730.

Journal of Dermatology (1989) **120**, 21–30.

25-dihydroxyvitamin D_3 increases intracellular free calcium in murine B16 melanoma

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SUMMARY

D_3 and its active metabolite 1α-25-dihydroxyvitamin D_3 (1α-25-$(OH)_2D_3$) have been to play a role in melanogenesis. Physiological concentrations of 1α-25-$(OH)_2D_3$ were acutely elevate intracellular free calcium (using Fura 2) in B16 primary (1°) cells. ne phosphoinositide turnover was unaffected by 1α-25-$(OH)_2D_3$. The rise in ular free calcium was entirely dependent on extracellular calcium and was not mimicked in D_3. However, in neither B16-1° nor B16-F_1 melanoma cells did vitamin D_3 or 1α- $)_2D_3$ increase melanin production.

reports have proposed that vitamin D metabolites are involved in UV induced enesis in human melanocytes and melanoma cells.[1,2] The mechanism of UVB-induced ation is itself not understood. UVB irradiation activates tyrosinase, the rate limiting in melanogenesis, but there is no evidence that it does so via cyclic AMP, the lar mediator used by melanocyte stimulating hormone (MSH) in the activation of e and production of melanin.[3]

however, is known to be involved in the biosynthesis of vitamin D_3, converting 7- cholesterol into pre-vitamin D_3 which then isomerizes to vitamin D_3 in the skin. There ce that vitamin D_3 itself stimulates melanogenesis in cultured human melanocytes[4] and ouse B16 melanoma C_3M subline.[5] This has led to the suggestion that one of the tes of vitamin D_3 is normally involved in the melanogenic response to UVB in the skin.[1] ld have important physiological and pharmaceutical implications. However, others d to confirm that either vitamin D_3 or its active metabolite, 1α-25-dihydroxy vitamin 25-$[OH]_2D_3$) affect melanogenesis in melanocytes.[6]

present study we have confirmed that B16 melanoma cells are capable of recognizing onding to physiological concentrations of 1α-25-$(OH)_2D_3$. 1α-25$(OH)_2D_3$ rapidly intracellular free calcium (within minutes) to an extent as great as or greater than that

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seen in response to 10μM A23187 (a potent calcium ionophore). This is the first report (OH)$_2$D$_3$ acutely elevating intracellular free calcium in B16 melanoma cells physiological implications of this are discussed.

1α-25(OH)$_2$D$_3$ and vitamin D$_3$ were, however, without effect on melanin producti melanoma cells.

METHODS

Materials

Eagles MEM, L-glutamine, penicillin/streptomycin, fungizone, 100 × vitamin co non-essential amino acids, sodium pyruvate, phosphate-buffered-saline and tryps were obtained from Flow Labs. Ayrshire, U.K. Fetal calf serum was obtained fro Europe Ltd, Paisley, U.K. Synthetic melanin (prepared by persulphate oxidation of α-melanocyte-stimulating-hormone and A23187 were obtained from Sigma Che Dorset, U.K. [^{3}H]-myoinositol was obtained from Amersham International Ltd, U. AM was obtained from Calbiochem (Behring Diagnostics), California, U.S.A. Tiss plastics were from Sterilin Limited, Feltham, U.K. Vitamin D$_3$ and 1α-25-dihydro D$_3$ were a gift from Shiseido Co. Japan. W$_7$ (N-(6-aminohexyl)-5-chloro-naph sulphonamide) was a gift from Dr G.M.Blackburn, Department of Chemistry, University, U.K.

Tissue culture

B16 primary (1°) melanoma cells were used at first passage from tumours raised mice.[7] Tumours were removed and homogenized using a glass hand homogenizer MEM with penicillin/streptomycin (40 IU/ml), fungizone (1·25 μg/ml), L-glutam sodium pyruvate (1 mM), 1·5% 100X vitamin concentrate, 1% non-essential-am NaHCO$_3$ (0·18%) and 10% fetal calf serum. Tumour homogenate was then seeded i tissue culture flasks and cells grown to confluence (1 to 2 weeks). Cells were pass 0·25% trypsin-EDTA solution and seeded into six well plates of 2·5 cm diameter at a 2·5 × 10^5 cells per well.

Measurement of melanin

Extracellular melanin present in the medium was measured spectrophotometrically The absorbance of the phenol-red containing Eagles MEM was found to change Accordingly, a standard curve of absorbance against medium pH was plotted to proportion of the total absorbance due to the pH of the medium to be calculated. The optical density was then used to calculate melanin concentration from a standar synthetic melanin (0·1–100 μg/ml) made up in 1 M NaOH. Intracellular melani measured spectrophotometrically after prior digestion of the cells in 1 M NaOH ov

To test the validity of this procedure for measuring melanin, recovery experi performed where a known amount of synthetic melanin was added to Eagles MEM amelanotic cell digest (in 1 M NaOH). The pH of the medium seeded with melanin wa prior to determination of medium absorbance at 400 nm. The optical density due alone was determined from the standard curve of medium pH against absorbance then subtracted from the total absorbance at 400 nm. The remaining absorbance determine the amount of melanin present from the standard curve of absorba melanin concentration (0·1–100 μg/ml). Melanin recovery from an amelanotic cell di with melanin was determined directly from the optical density at 400 nm. The recovery of melanin (10 μg/ml) from either medium or an amelanotic cell digest was found to be 95% (i.e. 9·5 μg/ml was found after correction for pH attributable absorbance.

Phosphatidyl inositol turnover

Measurement of phosphatidyl inositol (PI) turnover was based on the original method of Berridge *et al.*,[8] and adapted for use with cultured cells. Prior to incubation with vitamin D$_3$ or 1α-25-(OH)$_2$D$_3$, the medium was removed from the cultures and replaced with medium containing 0·1% bovine serum albumin (BSA) instead of 10% FCS and 1 μCi/well [^{3}H]-myoinositol for 24 h. After incubation, this medium was removed, and cells washed twice in standard EMEM. Cells were then preincubated in medium containing 10 mM lithium chloride and 0·5% BSA for 15 min at 37°C. Agonists were then added, and cells incubated for 1 h at 37°C. Incubations were terminated by removing the medium, adding 0·625 ml cold methanol to each well, scraping the cells with a 1 ml plastic syringe plunger, and then washing with a further 0·625 ml methanol. Both methanol extracts were combined and water soluble [^{3}H]-labelled inositol phosphates separated from organic [^{3}H]-labelled phospholipids, as described by Baird *et al.*[9]

Measurement of intracellular free calcium

B16-1° melanoma cells were grown as monolayers on glass coverslips (9 × 27 mm) and also used in suspension (10^6 cells/ml) (for the latter, cells were made into a suspension using Trypsin-EDTA 0·25%). Monolayers and cells in suspension were incubated with 4 μM Fura-2-AM for 30 min in balanced salt solution (BSS) (135 mM NaCl, 4·5 mM KCl, 1·5 mM CaCl$_2$, 0·5 mM MgCl$_2$, 5·6 mM glucose, and 10 mM Hepes, pH 7·4, in double distilled water), then rinsed in BSS and, in the case of monolayers, secured in a 1 cm quartz cuvette at an angle of 30° to the excitation beam. Cells were routinely excited at 335 nm, with a slit width of 5 nm, and emissions collected at 500 nm.[10] Changes in fluorescence were recorded using an Aminco-Bowman spectrophotofluorometer.

With cells in monolayers, we were unable to calibrate the fluorescence signal by the normal method of obtaining maximum and minimum fluorescence signals for Fura 2,[10] as detergents (digitonin, saponin, deoxycholate and triton) failed to permeabilize the cells. (It is our experience that B16 melanoma cells, and other cells in suspension cultures are readily permeabilized by detergents, e.g. 10 μM digitonin, but monolayers are not.) Accordingly, results are presented by qualitative comparison with the response to 10 μM calcium ionophore A23187.

Measurement of protein

Protein was measured by the method of Lowry *et al.*[11]

Statistics

The difference between means was assessed for significance by Student's *t*-test. Values of $P < 0{\cdot}05$ were taken as statistically significant.

RESULTS

Effects on intracellular free calcium

1α-25-(OH)$_2$D$_3$ increased intracellular free calcium in B16-1° cells. 1α-25-[OH]$_2$D$_3$ in the concentration range 10^{-10} to 10^{-9} M increased intracellular free calcium in all of nine subconfluent monolayers (approximately 4–5 × 10^5 cells per 2·5 cm diameter well), but in only five out of 15 confluent monolayers (1·5–2 × 10^6 cells). Vitamin D$_3$ itself, however, at equivalent

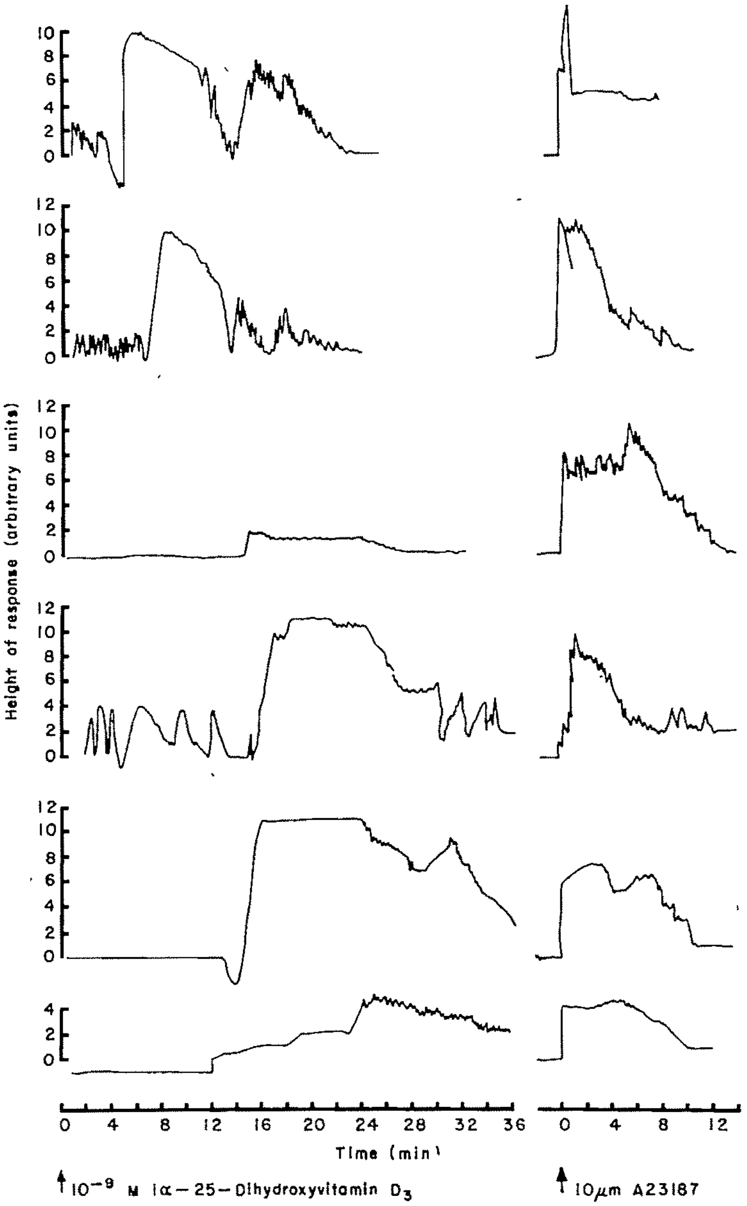

FIGURE 1. Effects of 1α-25-$(OH)_2D_3$ (10^{-9} M) and calcium ionophore A23187 (10 μM) on intracellular free calcium using Fura 2 in B16-1° melanoma monolayers. 1α-25-$(OH)_2D_3$ was added to the cuvette at time 0 as indicated and later 10 μM A23187 was added for comparison on the same monolayer. Results shown are of six different subconfluent monolayers. The *y*-axis shows arbitrary units of fluorescence where increasing height represents increasing intracellular free calcium (see text).

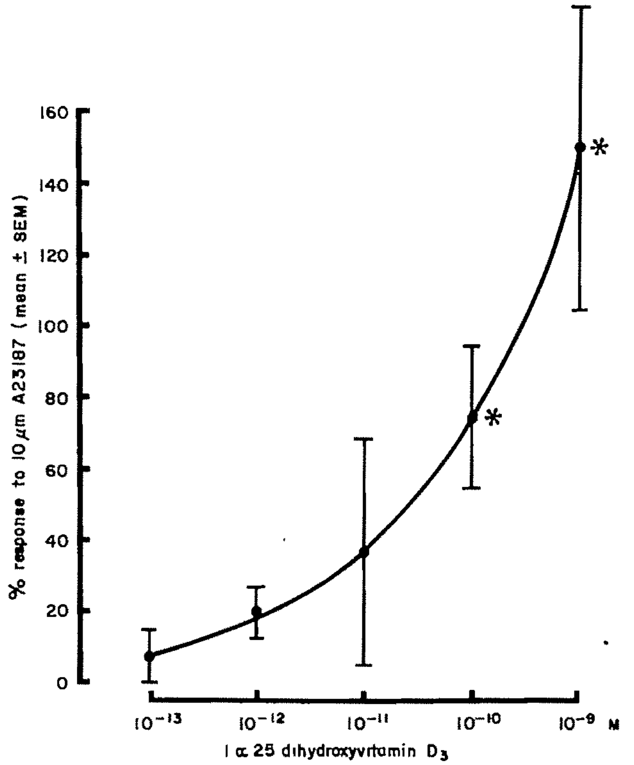

FIGURE 2. Effects of 1α-25-$(OH)_2D_3$ on intracellular free calcium in B16-1° melanoma. Results show the mean ± SEM of seven subconfluent monolayers in which the maximum height of the response to 1α-25-$(OH)_2D_3$ is expressed as a percentage of the maximum height of the response to 10 µM A23187 in the same monolayer. $^*P < 0{\cdot}05$ compared with intracellular free calcium in the absence of any agonist.

concentrations had no effect on intracellular free calcium in any of four subconfluent and seven confluent monolayers.

B16-1° cells in suspension (10^6 cells/ml) were not affected by 10^{-9} M 1α-25-$(OH)_2D_3$. In B16-1° cell suspensions, which are readily permeabilized, the resting level of intracellular free calcium was found to be 100 ± 48 nM ($n = 3$), rising to 1·5 ± 0·25 µM ($n = 3$) following addition to 10 µM A23187.

The increase in intracellular free calcium occurred 4–14 min after addition of 1α-25-$(OH)_2D_3$, in contrast to the response to the potent calcium ionophore A23187 (10 µM), which caused an immediate increase in intracellular free calcium (within 30 s) (see Fig. 1). The increase in intracellular free calcium in response to 1α-25-$(OH)_2D_3$ was as great or greater than the response to A23187 in each monolayer, and lasted 10–20 min before resting levels were re-attained.

Figure 2 shows a dose response curve to 1α-25-$(OH)_2D_3$ in which responses are expressed as a percentage of the response to 10 µM A23187 in the same monolayer. Results based on seven different monolayers are shown. Despite the considerable variation from monolayer to monolayer it is clear that at 10^{-9} M 1α-25-$(OH)_2D_3$ the increase in intracellular free calcium was as great or greater than the response to 10 µM A23187.

The response to 1α-25-$(OH)_2D_3$ was entirely dependent on the presence of extracellular

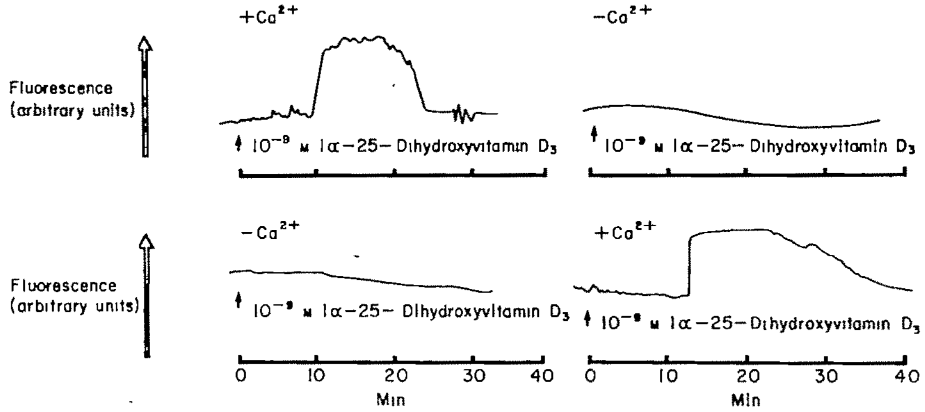

FIGURE 3. The effect of extracellular calcium on the ability of 1α-25-$(OH)_2D_3$ to elevate intracellular calcium. 1α-25-$(OH)_2D_3$ (10^{-9} M) was added to the cuvette both in the presence and absence of extracellular calcium in the BSS. The upper panel shows a response to 1α-25-$(OH)_2D_3$ in a monolayer in BSS containing calcium. This was then changed to calcium free BSS and the response to 1α-25-$(OH)_2D_3$ was lost. The lower panel shows another monolayer in which 1α-25-$(OH)_2D_3$ was first examined in BSS without calcium and then BSS plus calcium.

calcium. If calcium was absent from the BSS, the response to 1α-25-$(OH)_2D_3$ was lost, but could be restored by re-addition of extracellular calcium, as illustrated in Figure 3.

Effects of phosphotidyl inositol hydrolysis

Activation of membrane phosphoinositide hydrolysis can result in an acute transient increase in intracellular free calcium. Vitamin D_3 and 1α-25-$(OH)_2D_3$ however, had no consistent or

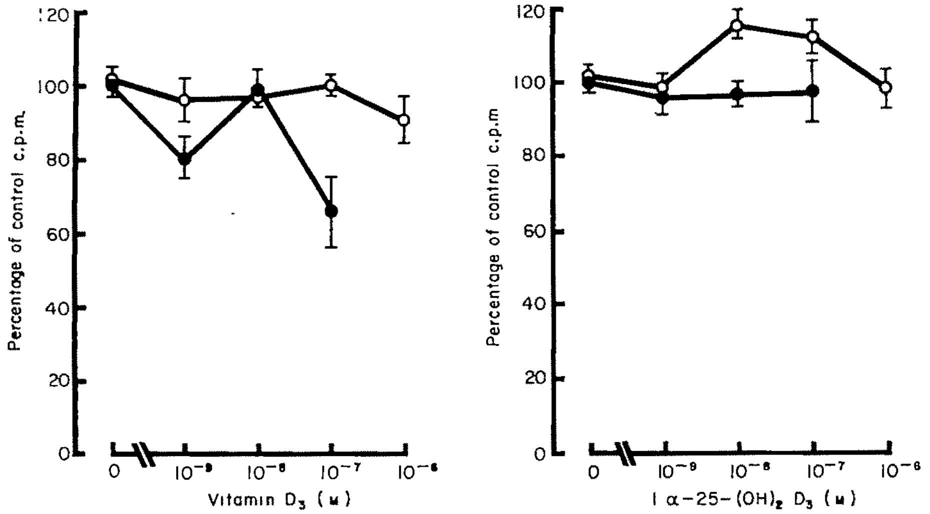

FIGURE 4. Effects of vitamin D_3 and 1α-25-$(OH)_2D_3$ on B16-1° melanoma phosphatidyl inositol turnover. In two separate experiments (○ and ●) vitamin D_3 and 1α-25-$(OH)_2D_3$ were incubated with B16-1° cells for 1 h in the presence of 10 mM LiCl, and the rate of [^{3}H] inositol phosphate accumulation determined. Values are means ± SEM of triplicate determinations expressed as a percentage of the c.p.m. (counts per minute) obtained in control cultures.

TABLE 1. Effects of vitamin D_3, 1α-25-dihydroxyvitamin D_3 and W_7 on melanin production in B16-1° and F_1 melanoma cells. Cells were incubated for 5 days with the agents shown and melanin production measured in both medium and cells. Results shown are of a single representative experiment

Additions	Total melanin (μg/mg protein)	
	B16-1°	B16-F_1
None (control)	3·13 ± 0·87	18·50 ± 5·00
Vitamin D_3, 10^{-9} M	2·48 ± 1·40	20·00 ± 0·10
1α-25-$(OH)_2D_3$, 10^{-9} M	3·00 ± 0·90	15·40 ± 2·90
W_7, 25 μM	9·50 ± 1·00*	40·00 ± 1·00*

* Significantly greater than control $P < 0·05$.

significant effect on PI hydrolysis in B16 melanoma 1° cells (Fig. 4) ($P > 0·05$). It was interesting to note that the rate of PI hydrolysis in B16-1° cells was 42% of the total [^{3}H]-labelled inositol phosphates within the cell in 1 h in the absence of any added agonist. By comparison, other non-neoplastic cell lines in our laboratory have a turnover in 1 h of only 2–5%.

Effects on melanogenesis

Neither vitamin D_3 nor 1α-25-$(OH)_2D_3$ over a wide range of concentrations had any effect on melanin production in B16-1° or F_1 cells. The results are summarized in Table 1 where the total

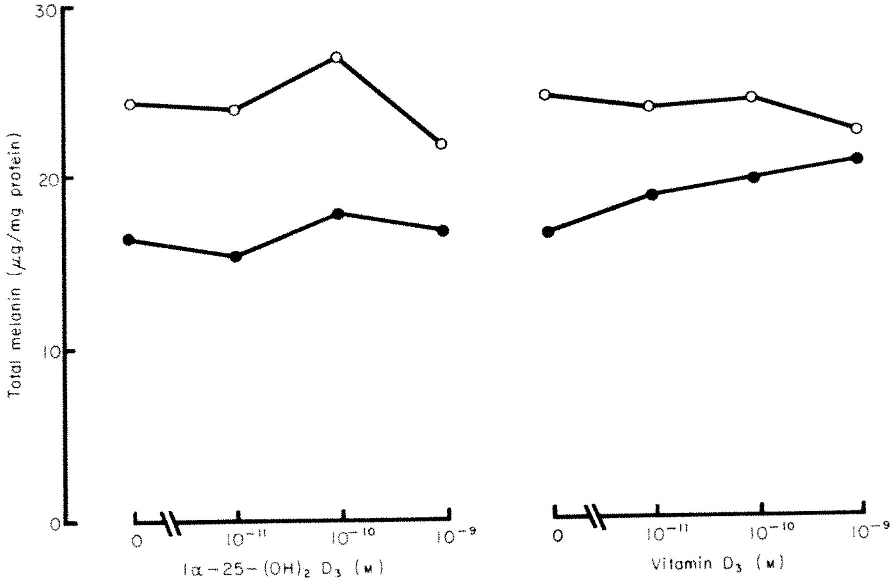

FIGURE 5. Effect of vitamin D_3 and 1α-25-$(OH)_2D_3$ with or without αMSH on total melanin production in B16-F_1 melanoma. Cells were incubated for 5 days in medium with either vitamin D_3 or 1α-25-$(OH)_2D_3$ alone (●) or in the presence of 10^{-7} MαMSH (○). Medium was replaced on day 3, and total melanin (in medium from days 3 and 5 and within the cells) was determined. Results shown are of a single representative experiment.

melanin production of cells incubated with 10^{-9} M vitamin D_3 or 1α-25-$(OH)_2D_3$ for 5 days is shown. By contrast, the calmodulin antagonist W7 significantly increased melanogenesis in both cell lines ($P < 0.05$).

Vitamin D_3 and 1α-25-$(OH)_2D_3$ also failed to affect α MSH stimulated melanogenesis in B16 F_1 cells (Fig. 5). B16-1° cells, unlike B16 F_1 cells do not pigment in response to α MSH as we have previously reported.[19]

DISCUSSION

In the present study we have demonstrated that 1α-25-$(OH)_2D_3$ dramatically elevates intracellular free calcium in B16 melanoma primary cells to levels as high as would ever be experienced in cells (1–2 μM).

The increase in intracellular calcium occurred within a few minutes of addition of 1α-25-$(OH)_2D_3$ to B16 melanoma monolayers and then persisted for 10–20 min. This response time is very unusual and difficult to reconcile with current knowledge of 1α-25-$(OH)_2D_3$ action. Also, although an increase in intracellular free calcium can occur either by release of calcium from intracellular calcium stores or influx of extracellular calcium, the effects of 1α-25-$(OH)_2D_3$ in the present study could be explained entirely by influx of extracellular calcium—no effect of 1α-25-$(OH)_2D_3$ was demonstrable on phosphatidyl inositol hydrolysis and removal of extracellular calcium prevented the response to 1α-25-$(OH)_2D_3$.

Soluble receptors for 1α-25-$(OH)_2D_3$ have now been found in a variety of cell types other than those tissues classically considered to be the physiological targets for 1α-25-$(OH)_2D_3$, for example B16 melanoma,[1] human keratinocytes,[12] rat osteogenic carcinoma,[13] human breast cancer T47-D cells[14] and human leukaemic HL-60 cells.[15] Binding of 1α-25-$(OH)_2D_3$ to these receptors is followed by induction of RNA synthesis in the nucleus leading to the production of a calcium binding protein which is then thought to facilitate calcium entry into the cell, the major function of 1α-25-$(OH)_2D_3$. This process takes several hours. However, other workers have noted much more acute effects of 1α-25-$(OH)_2D_3$ on calcium transport.[16,17] For example, Bikle *et al.*[16] reported that cyclohexamide did not block 1α-25-$(OH)_2D_3$ induced calcium transport in intestinal epithelium despite being able to block 1α-25-$(OH)_2D_3$ induced calcium binding protein production. Also, Fontaine *et al.*[18] reported that 1α-25-$(OH)_2D_3$ altered lipid composition and so calcium permeability of rat intestinal membranes.

In the present study we found 1α-25-$(OH)_2D_3$ elevated intracellular free calcium (probably to levels as high as would ever be experienced in the living cell[10]) within a few minutes. This time course is unlikely to be compatible with 1α-25-$(OH)_2D_3$ inducing synthesis of a calcium binding protein. Also, we failed to find any effect of 1α-25-$(OH)_2D_3$ on suspensions of B16-1° cells. As damage to cell surface receptors and proteins often occurs when monolayers of cells are made into suspensions, this suggests that 1α-25-$(OH)_2D_3$ needs to interact with some membrane structure for these acute effects. The mechanism by which 1α-25-$(OH)_2D_3$ elevates intracellular calcium obviously requires much further study; in the present study we have shown that it causes an influx of extracellular calcium rather than a phosphoinositide-induced release of intracellular calcium.

Interestingly, 1α-25-$(OH)_2D_3$ was more likely to elevate intracellular free calcium in sub-confluent monolayers than in fully confluent monolayers. One might speculate that the characteristics and composition of the cell surface may change as cells become more mature. This maybe a relevant parameter to consider when investigating the effects of 1α-25-$(OH)_2D_3$ on melanogenesis. B16 melanoma cells differ markedly in their melanogenic and proliferative behaviour. In the present study, B16-F1 and primary cells reached confluence 1–2 days following the start of incubation with 1α-25-$(OH)_2D_3$. It is possible that they then lose the ability to respond to 1α-25-$(OH)_2D_3$.

Our results with 1α-25-$(OH)_2D_3$ contrast with those of Hosoi *et al.*[1] who found that 1α-25-$(OH)_2D_3$ increased melanogenesis in B16 melanoma. However, it is possibly relevant that the methodologies used were different. Hosoi *et al.*[1] examined the ability of 1α-25-$(OH)_2D_3$ to produce pigmented colonies of B16 melanoma cells in agar, unlike the present study of B16 melanoma cells in monolayers. Under these standard tissue culture conditions, adequate for MSH (in the case of B16-F_1 cells in the present study) or UV-induced melanogenesis (as in the study by Mansur *et al.*[6]) vitamin D_3 and 1α-25-$(OH)_2D_3$ do not affect melanogenesis.

The effects of 1α-25-$(OH)_2D_3$ on both intracellular free calcium and melanogenesis obviously require further investigation in other melanogenic cells in order to establish whether the increase in calcium may be relevant to the reported melanogenic response. B16 melanoma cells themselves differ markedly in their ability to produce melanin, either spontaneously or in response to stimulation by αMSH[19] or UVB irradiation (unpublished observations). For example, in our hands B16 melanoma 1° cells, derived from pigmented tumours, will melanize in response to calmodulin antagonists yet fail to do so in response to αMSH. This is despite an αMSH-stimulated elevation of intracellular cyclic AMP which is as great as that seen in the other αMSH responsive B16 melanoma lines.[19]

Even in cells where 1α-25-$(OH)_2D_3$ is reported to induce melanin production[4,20] the mechanism by which it does so is not known. Hosoi *et al.*[1] found 1α-25-$(OH)_2D_3$ and vitamin D_3 to be without effect on cyclic AMP production, yet 1α-25-$(OH)_2D_3$ stimulated melanogenesis.

It is now clear that the rate-limiting enzyme in melanogenesis, tyrosinase, can be activated by both cyclic AMP-dependent and independent mechanisms. The former is well documented (for example by Halaban *et al.*,[21] but cyclic AMP does not seem to be involved in the activation of tyrosinase which occurs in response to UVB irradiation,[3] 1α-25-$(OH)_2D_3$, retinoids[1,22] or calmodulin antagonists.[7,23]

The other major intracellular mediator which is involved in both cell proliferation and differentation is calcium. A small transient rise in intracellular free calcium can act as a signal in cell proliferation and in many differentiated cell functions. Sustained high levels of intracellular free calcium lead to cell death.[24] It is possible that UVB irradiation, 1α-25-$(OH)_2D_3$, retinoids and calmodulin antagonists may all in some way modulate intracellular free calcium and either directly or indirectly affect tyrosinase activity. The role of calcium and calmodulin in the regulation of melanogenesis has not been investigated.

Thus, contrary to previous reports,[1] we did not observe any melanogenic response to 1α-25-$(OH)_2D_3$ in B16 melanoma 1° or F_1 cells. However, we report, for the first time, that B16 melanoma 1° cells do have the means of recognizing and responding to 1α-25-$(OH)_2D_3$ with an unexpectedly large and rapid increase in intracellular free calcium. In these cells the physiological significance of this response to 1α-25-$(OH)_2D_3$ is not clear. *In vivo* these cells form pigmented tumours. *In vitro* they pigment in response to calmodulin antagonists, but not in response to MSH. As our current understanding of the control of melanogenesis points strongly to both a cyclic AMP dependent and a cyclic AMP independent means of activating tyrosinase, we suggest that an investigation of the role of intracellular calcium in the regulation of melanogenesis could clarify many of the unanswered questions in this area.

Irrespective of any effects of 1α-25-$(OH)_2D_3$ on melanogenesis, the acute effects of this steroid on intracellular calcium which we describe may represent an important contribution to our understanding of the cellular mechanism of action of 1α-25-$(OH)_2D_3$.

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REFERENCES

1 Hosoi J, Abe E, Suda T, Kuroki T. Regulation of melanin synthesis of B16 mouse melanoma cells by 1α-25-dihydroxyvitamin D_3 and retinoic acid. *Cancer Res* 1985; **45:** 1474–8.

2 Tomita Y, Torinuki W, Tagami H Stimulatory effect of vitamin D_3 on cultured human melanocytes. A possible mechanism underlying pigmentation after UV irradiation (Abstract). *J Invest Dermatol* 1987; **89:** 289–301.

3 Friedmann PS, Gilchrest BA. UV irradiation directly induces pigment production by cultured human melanocytes. *J Cell Physiol* 1987; 133: 88–94.

4 Tomita Y, Torinuki W, Tagami H. Stimulation of melanogenesis by cholicalciferol in cultured human melanocytes: A possible mechanism underlying pigmentation after ultraviolet irradiation. *Tohuku J Exp Med*, 1986; **149:** 451–2.

5 Oikawa A, Nakayasu M. Stimulation of melanogenesis in cultured melanoma cells by calciferols. *FEBS Letts* 1974; **42**(1): 32–5.

6 Mansur CP, Gordon P, Holick MF, Gilchrest BA. Vitamin D does not affect melanisation of cultured human melanocytes (Abstract). *J Invest Dermatol* 1988; **91:** 16–21.

7 MacNeil S, Walker SW, Senior HJ *et al.* Effects of extracellular calmodulin and calmodulin antagonists on B16 melanoma cell growth. *J Inv Dermatol* 1984; **83:** 15–19.

8 Berridge MJ, Downes CP, Hanley MR. Lithium amplifies agonist dependent phosphatidylinositol responses in brain and salivary glands. *J Biochem* 1982, **206:** 587–95.

9 Baird JG, Dobson PRM, Wojcikiewicz RJH, Brown BL. Thyrotropin-releasing-hormone stimulates inositol-phosphate production in normal anterior pituitary cells and GH_3 tumour cells in the presence of Li^+. *Biosci Reports* 1983; **3:** 1091–9.

10 Tsien TY, Rink TJ, Poenie M. Measurement of cytosolic free Ca^{2+} in individual small cells using fluorescent microscopy with dual excitation wavelengths. *Cell Calcium* 1985, **6:** 145–57.

11 Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with Folin phenol reagents. *J Biol Chem* 1951; **193:** 265–75.

12 Hosomi J, Hosoi J, Abe E *et al.* Regulation of terminal differentiation of cultured mouse epidermal cells by 1α-25-dihydroxyvitamin D_3. *Endocrinology* 1982, **113:** 1950–7.

13 Dokoh S, Donaldson CA, Haussler MR. Influence of 1α-25-dihydroxyvitamin D_3 on cultured osteogenic sarcoma cells: correlation with the 1α-25-dihydroxyvitamin D_3 receptor. *Cancer Res* 1984; **44:** 2103–9.

14 Sher E, Eisman, JA, Moseley JM, Martin TJ. Whole cell uptake and nuclear localisation of 1α-25-dihydroxyvitamin D_3 by cancer cells (T47-D) in culture. *J Biochem* 1981; **200:** 315.

15 Mangelsdorf DJ, Koeffler HP, Donaldson CA *et al.* 1α25-dihydroxyvitamin D_3-induced differentiation in a human promyelocytic leukemic cell line (HL60), receptor mediated maturation to macrophage-like cells *J Cell Biol* 1984; **98:** 391–8.

16 Bikle DD, Zolock DT, Morrissey RL. Action of vitamin D on intestinal calcium transport. *Ann NY Acad Sci* 1981; **V:** 481–500.

17 Desai SS, Appel MC, Baran D-T. Differential effects of 1α-25-dihydroxyvitamin D_3 on cytosolic calcium in two human cell lines (HL-60 and U937). *J Bone Mineral Res* 1986; 1(6): 497–501.

18 Fontaine O, Matsumoto T, Goodman DBP, Rasmussen H. Liponomic control of the Ca^{2+} transport. Relationship to mechanism of action of 1α-25-dihydroxyvitamin D_3. *Proc Natl Acad Sci USA* 1981; **78**(3): 1751–4.

19 Hill SE, Thody AJ, Oliver I *et al.* Investigation of the regulation of pigmentation to αMSH and W_7 in B16 melanoma cell lines. *Pigment Cell Res* 1988; **1:** 292.

20 Kuroki T, Chida K, Hashiba H *et al.* Regulation of cell differentiation and tumour promotion by 1α-25-dihydroxyvitamin D_3. Carcinogenesis (E Huberman, SH Barr, eds), Vol. 10, New York: Raven Press, 1985; 275–86.

21 Halaban R, Pomerantz SH, Marshall S, Lerner AB. Tyrosinase activity and its abundance in Cloudman melanoma cells. *Arch Biochem Biophys* 1984; **230:** 383–7.

22 Lotan R, Lotan D. Enhancement of melanotic expression in cultured mouse melanoma cells by retinoids. *J Cell Physiol* 1981; **106:** 179–89.

23 MacNeil S, Walker SW, Senior HJ *et al.* Calmodulin activation of adenylate cyclase in B16 melanoma. *J Biochem* 1984; **224:** 453–60.

24 Tomlinson S, MacNeil S, Walker SW *et al.* Calmodulin and cell function. *Clin Sci* 1984; **66:** 497–508.

British Journal of Dermatology (1989) **120,** 31–36.

Bone-scintigraphic examinations in patients treated with retinoids: a prospective study

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SUMMARY

Possible early side-effects of retinoid treatment on bones were studied with the aid of bone-scintigraphy. Isotretinoin treatment (1·0 mg/kg daily for 4 months) was given to 18 patients with acne. Fifteen patients with psoriasis received etretinate treatment (0·7–1·0 mg/kg/day) for 4 months. In the group treated with isotretinoin, pathological uptake of radiolabel was found in three cases, while in the group treated with etretinate, no bone changes attributable to treatment were found. During isotretinoin treatment, a decrease in growth-plate activity was observed. Bone-scintigraphy is considered to be a suitable method for early screening for bone changes occurring in retinoid treatment.

Over the last decade the introduction of the oral retinoids isotretinoin and etretinate has brought great advances in dermatological therapy. At present, problems concerning the efficacy of treatment and chronic toxicity are the centre of interest. Recently published reports suggest that side effects on the skeletal system—observed after long-term treatment with high doses of retinoid—are of importance.[1,2]

We wished to establish whether retinoids can be considered safe with regard to the skeletal system, when given in moderate doses for some months. Therefore, we performed prospective bone-scintigraphic examinations—which are more sensitive than X-ray investigations—in patients treated with etretinate and isotretinoin.

METHODS

Fifteen male patients with stable chronic psoriasis without symptoms of arthropathy (mean age 33 years, range 19–40 years) received etretinate treatment. After giving informed consent, they were given 0·7–1·0 mg/kg Tigason® daily for 4 months. Eighteen men with severe acne conglobata (mean age 22 years, range 16–42 years) received isotretinoin treatment, 1·0 mg/kg/day Roaccautane®, for 4 months. Bone-scintigraphic examinations were performed using

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Technetium 99^{m}-labelled phosphone, with 550 MBq given by i.v. injection. Three hours after the injection, 300,000 count images were produced on X-ray film anterior to the knees and posterior to the sacroiliac joints. If the growth plate was open, its activity was expressed relative to the femur (ratio of activity of a unit-area of open growth plate activity : a unit area of the femur). Activity of the sacroiliac joints was expressed relative to the middle of the sacrum, using computer analysis. Scintigraphic examinations were performed in all patients 0–4 weeks before and at the end of treatment. Examinations were completed by calcium and phosphorus determinations. Statistical analysis was carried out using Student's *t*-tests.

RESULTS

In 15 of the 18 acne patients treated with isotretinoin, no changes were observed after treatment as compared to the baseline films. In the other three patients, various degrees of increased activity were observed. In one case a marked increase was seen in the medial condyles of the knee-joints and in the sacroiliac joint (Fig. 1). In the second case there was a low-level uneven increase in activity in the projection of the left patella and the articular space, while in the third case we observed a circumscribed increase in activity in the middle of the left knee.

Evaluating data from seven patients with open growth plates by computer analysis, we found a significant decrease in mean relative activity (Table 1). There was also a decrease in mean relative activity of the sacroiliac joints in 15 patients (Table 2). Before treatment, however, four patients had higher values attributable to unilateral or bilateral sacroileitis,[1,3] although after treatment the activity ratios fell below 1·3 in every case. In 12 of the 15 psoriasis patients treated with etretinate no changes relative to the baseline films could be observed after treatment. In three cases increased uptake of radiolabel was noted. In two cases slightly increased activity located at the distal end of the femur was already seen on the baseline film. In the third case, pathological accumulation was evident in projection of the right patella, but here again activity was slightly uneven in the proximal region of the tibias before treatment.

On the basis of relative activity, there was unilateral sacroileitis in two patients before

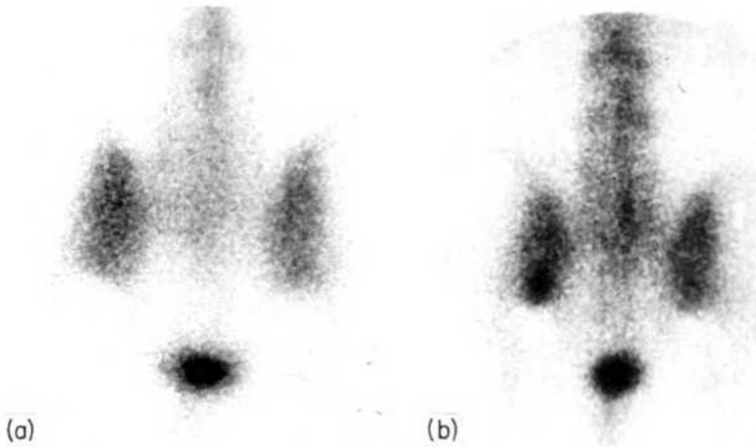

FIGURE 1. Patient 18. Posterior lumbosacral films (a) before isotretinoin treatment and (b) after isotretinoin treatment showing increase in activity in the distal region of the left sacroiliac joint.

TABLE 1. Activity of growth-plate before and after isotretinoin treatment in seven patients with open growth plates. Values are ratios of activity of growth plate: activity of femur

Patient no.	Before treatment		After treatment	
	Left knee	Right knee	Left knee	Right knee
20	2·94	3·02	2·02	1·83
21	3·47	3·50	1·92	1·92
23	2·40	2·07	2·09	1·86
24	3·50	3·01	2·37	2·44
25	3·30	3·41	1·55	1·53
29	4·34	3·81	2·57	2·44
31	3·66	3·11	2·57	2·46
mean ± SEM	3·37 ± 0·21	3·13 ± 0·19	2·16 ± 0·13*	2·07 ± 0·13*

* Significantly less than pretreatment value $P < 0{\cdot}01$.

treatment, the activity ratio decreasing by the end of treatment to below 1·3. Mean values for left and right sacroiliac activity, calculated in 11 cases, showed a slight decrease after treatment (Table 3). Patients with increased uptake had no subjective symptoms and clinical examination did not reveal any locomotor disorder.

Serum calcium and phosphorus levels increased slightly in the etretinate group; the rise in

TABLE 2. Activity of sacroiliac joints before and after isotretinoin treatment in 15 patients. Values are ratios of activity of sacroiliac joint: activity of middle of sacrum

Patient no.	Before treatment		After treatment	
	Left side	Right side	Left side	Right side
17	1·17	1·24	1·05	1·11
19	1·04	1·08	1·08	1·14
20	1·29	1·30	1·12	1·12
21	1·40*	1·47*	1·20	1·27
22	1·07	1·18	1·04	1·04
23	1·12	1·20	1·11	1·18
24	1·12	1·18	1·03	1·12
25	1·17	1·32*	1·09	1·18
27	1·10	1·13	1·07	1·19
28	1·47*	1·50*	1·14	1·28
29	1·28	1·17	1·12	1·11
30	1·27	1·24	1·18	1·23
31	1·25	1·28	1·26	1·38
32	1·00	1·47*	1·20	1·14
33	1·04	1·11	1·02	1·06
mean ± SEM	1·18 ± 0·034	1·25 ± 0·033	1·11 ± 0·018**	1·17 ± 0·022**

* Sacroileitis.

** Significantly less than pretreatment value $P < 0{\cdot}05$.

TABLE 3. Activity of sacroiliac joints before and after etretinate treatment in 11 patients. Values are ratios of activity of sacroiliac joint: activity of middle of sacrum

Patient no.	Before treatment		After treatment	
	Left side	Right side	Left side	Right side
1	1·03	1·21	1·10	1·15
2	1·15	1·15	1·12	1·24
3	1·10	1·20	1·05	1·15
4	1·07	1·26	1·08	1·28
5	1·10	1·15	0·98	0·99
6	1·27	1·22	1·00	1·07
7	1·12	1·15	1·14	1·10
8	1·19	1·27	1·09	1·09
9	1·29	1·36*	1·18	1·16
10	1·18	1·25	1·18	1·16
11	1·30	1·46*	1·04	1·07
mean ± SEM	1·16 ± 0·26	1·24 ± 0·27	1·09 ± 0·19**	1·13 ± 0·24†

* Sacroileitis. Significantly lower than pre-treatment value.
** $P<0\cdot05$, † $P<0\cdot02$.

TABLE 4. Serum calcium and phosphorus levels before and after treatment with isotretinoin or etretinate. Values are means ± SEM

	Etretinate		Isotretinoin	
	Calcium (nmol/l)	Phosphorus (nmol/l)	Calcium (nmol/l)	Phosphorus (nmol/l)
Before treatment	2·31 ± 0·04	1·05 ± 0·12	2·38 ± 0·03	0·89 ± 0·06
After treatment	2·43 ± 0·02	1·39 ± 0·10	2·32 ± 0·03	0·99 ± 0·05
P	<0·05	NS	NS	NS

serum calcium was statistically significant. In the group treated with isotretinoin, serum calcium and phosphorus levels remained practically unchanged (Table 4).

DISCUSSION

Bone-scintigraphy complemented by computer controlled high-resolution gamma camera techniques makes it possible to study changes in the metabolic processes occurring in bones at a very early stage. Since the method is very sensitive, bone changes can be recognized which are not demonstrable by traditional radiological measures. A further advantage is unambiguous documentation of pathological bone-processes. Bone-scintigraphy is applicable to the diagnosis of inflammatory, traumatic, degenerative and neoplastic changes and also to those of metabolic origin. It can demonstrate increased turnover processes occurring in the bone, from which increased osteoblastic or osteoclastic activity may be concluded. Osteotropic radiolabel uptake demonstrated by bone scintigraphy is associated with local bone–blood circulation, mineralization of the matrix and generally with regeneration of bone and cartilage.[3]

Bone changes as a side-effect of long-term retinoid treatment at high doses were first described by Pittsley and Yoder in 1983.[4] These observations were subsequently confirmed by other authors.[5,6,7] The effect may be rarer after etretinate treatment than after treatment with isotreinoin and generally occurs after a longer treatment period with etretinate (> 4 years),[1,8–11] while isotretionoin can produce increased ossification after 32 weeks. X-ray investigations have demonstrated the following effects in connection with retinoid treatment: premature epiphyseal closure, increased transparency of the long tubular bones, thinning of long tubular bones, calcification of vertebral ligaments and tendons, osteoporosis and cortical hyperostisis.[7] While etretinate tends to produce increased calcification of the peripheral bones and ligaments, isotretinoin has a similar effect on spinal bones and ligaments.[7]

Bone-scintigraphic examinations enabled us to demonstrate increased uptake of radiolabel at the end of treatment in three of 18 patients with acne, who had been treated with isotretinoin. At the sacroiliac joint and patella, an increase in activity was observed in one instance, and in the medial part of the knee joints in two instances. In one case this increase was pronounced, while in the other two it was moderate. Clinically, the bone changes remained asymptomatic. Unfortunately, we could not carry out further bone examinations in these patients.

Bone-scintigraphy is a very sensitive, but relatively nonspecific examination. Evaluation of the picture must take into account a number of factors, including increased weight and injuries. Since we were unable to demonstrate any other factors likely to be responsible for bone changes in our patients, the increases in activity may be considered to be associated with isotretinoin treatment.

Computer monitoring showed an unambiguous decrease in activity of growth plates during isotretinoin treatment. Is is well known from X-ray investigations that earlier closure may occur on retinoid treatment. However, our present results do not permit us to conclude for certain that isotretinoin decreased growth-plate metabolism, as in our patients age was probably a factor in the closure of growth-plates. To settle this problem, an untreated control group of the same age would need to be investigated. It is interesting to note that in both groups of patients activity values indicative of sacroileitis had returned to normal after treatment, probably because of the anti-inflammatory effect of the retinoids.

In psoriasis patients treated with etretinate, we did not observe any pathological uptake of radiolabel that could have been attributed to treatment. In three of our patients we had already seen varying degrees of increased activity at baseline, extending to other bones after treatment. These results are similar to the recent report by Glover *et al.*[12] who did not find any scintigraphic changes in 19 patients receiving long-term etretinate treatment. In a high percentage of patients with psoriasis, pathological uptake was demonstrable by scintigraphy in the absence of clinical symptoms or changes detectable by X-ray. This can be interpreted as an extracutaneous osseous manifestation of psoriasis, attributable to increased collagen formation analogous to increased epidermopoiesis.[13] It is notable that we found a slight but significant increase in serum calcium in this group of patients.

On the basis of our investigations we believe that a relatively early increase in metabolic activity in bone is to be expected after administration of isotretinoin. On examination with extremely sensitive methods, in some cases this is already manifest four months after commencement of therapy. With etretinate treatment at the same stage bone changes could not be demonstrated. A decrease in growth-plate activity attributable to isotretinoin can only be confirmed by a controlled study, to exclude the influence of other factors. Scintigraphic examinations may be of importance in pinpointing areas where X-ray examination should be performed. In isotretinoin treatment of longer than 6 months' duration twice-yearly bone scans are to be recommended; these involve a smaller total radiation dose than a whole-body skeletal survey.

REFERENCES

1 Bennet P, Wigneron AM, Kaplan G. Poussee evolutive d'une hyperostose vertebrale ankylosante induits par un retinoide. *Presse Med* 1985; **14**: 596.

2 Török L, Budai S, Kása M, Kádár L. Bone X-ray examinations in patients treated with isotretinoin: a prospective study. *Br J Dermatol* 1988; **118**: 134–5.

3 Schumichen C. Grundlagen der Skelett- und Weichteilszcintigraphie. In: *Dermatologie und Nuklearmedizin* (Holzmann H, Altmeyer P, Hor G, Hahn K, eds). Berlin, Heidelberg, New York, Tokyo: Springer-Verlag, 1985; 15–25.

4 Pittsley RA, Yoder FW. Retinoid Hyperostosis. *New Engl J Med* 1983; **308**: 1012–14.

5 DiGiovanna JJ, Peck GL. Oral synthetic retinoid treatment in children. *Pediatric Dermatol* 1983; **1**: 77–8.

6 Ellis CN, Pennes DR, Martel W, Voorhees JJ. Radiographic bone surveys after isotretinoin therapy for cystic acne. *Acta Dermatovenereol* 1985; **65**: 83–5.

7 McGuire J, Milstone L, Lawson J. Isotretinoin adminsitration alters juvenile and adult bone. In: *Retinoids: New Trends in Research and Therapy* (Saurat JH, ed.). Basel, München: Karger, 1985; 419–39.

8 Burge S, Ryan T. Diffuse hyperostosis associated with etretinate. *Lancet* 1985; **ii**: 397–8.

9 Frenk E. Traitement oral de l'ichthyose lamellaire (erythrodermie ichthyosiforme congenitale non bulleuse) avec un retinoide arometique. *Dermatologica* 1981; **162**: 91–103.

10 Gerber LH, Helfgott RK, Gross EG, Hicks JE *et al.* Vertebral abnormalities associated with synthetic retinoid use. *J Am Acad Dermatol* 1984; **10**: 817–23.

11 Tsambaos D, Hilt K, Goos M. Knochenveranderungen unter Behandlung mit oralen synthetischen Retinoiden. *Akt Dermatol* 1985; **11**: 85–7.

12 Glover MT, Peters AM, Atherton DJ. Surveillance for skeletal toxicity in children treated with etretinate. *Br J Dermatol* 1987; **116**: 609–14.

13 Hahn K, Thiers G, Eissner D. Knochen- und Gelekszintigraphische Befunde bei Psoriasis. In: *Dermatologie und Nuklearmedizin* (Holzmann H, Altmeyer P, Hör G, Hahn K, eds). Berlin, Heidelberg, New York, Tokyo: Springer-Verlag, 1985: 26–35.

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Molluscum contagiosum virus types in genital and non-genital lesions

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SUMMARY

The endonuclease digest patterns of viral DNA from 48 genital and 45 non-genital molluscum contagiosum virus (MCV) lesions were examined. The overall ratio of MCVI to MCVII was 3·23:1. There was no predominance of either MCV type in genital lesions. No obvious morphological differences were seen between MCVI and MCVII lesions. MCVII was not found in any patient under 15 years old.

Two viruses that commonly infect the skin, herpes simplex virus (HSV) and human papilloma virus (HPV), have multiple strains—two in the case of HSV and numerous in the case of HPV. In the case of both viruses the clinical features may to a certain extent be related to the strain. Thus, in HPV it has been shown that certain strains are preferentially associated with skin warts of a particular morphology, with lesions at particular sites and with lesions with malignant potential.[1,2] Of the two strains of HSV, HSV1 is the predominant serotype in the common recurring facial lesions and in the rare encephalitis, whereas HSV2 is most frequently associated with genital infections of adults and less frequently with generalized infections of neonates.[3] In neonatal HSV encephalitis, infants infected with HSV2 fare worse than those infected with HSV1.[4]

Molluscum contagiosum virus (MCV) infection is another common virus infection of the skin and at least two different strains of the virus have been identified. As is the case with HSV and HPV, sexual transmission of MCV undoubtedly occurs. This is evident from the fact that lesions can be confined to the genital area, that MCV infection is prevalent in sexually active individuals, that it occurs in sexual partners and that its incidence is rising in common with other sexually transmitted diseases.[5,6]

In earlier reports of MCV DNA analysis, MCVII was the only virus type found in genital lesions.[7–9] In a subsequent study in which there were 12 MCVII samples there was no

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indication that either MCV subtype was predominantly associated with any particular site.[10] We have enlarged this last study to include 93 patients with MCV infection to determine whether the MCV sub-types are related to the site of infection or the clinical features of the lesions.

METHODS

Patients

Patients presenting to either the Skin or Genito-Urinary clinics were studied. Those with AIDS or known to be at risk of HIV infection were excluded and because one of our Genito-Urinary clinics was known to have a high proportion of homosexuals amongst its attenders the majority of genital samples were obtained from females.

Typing of MCV

Lesions were curetted or the virus-rich core expressed. DNA was recovered after deproteinization and cleaved with sequence-specific endonuclease BamHI or HindIII. The virus DNA fragments were separated by agarose gel electrophoresis and the cleavage patterns, which are type specific, were examined.[11]

Statistical analysis

The results were analysed using the Chi-squared test with Yates' correction.

RESULTS

The results are summarized in Tables 1 and 2. Ninety three samples, from various sites, were obtained from 93 patients, aged from 3 to 70 years. Fifteen patients were under 15 years old. Eight patients had atopic dermatitis.

Two endonuclease cleavage patterns of MCV DNA were demonstrated. Minor variations between isolates were evident in both patterns, but there was no difficulty in identifying them as either type I (MCVI) or type II (MCVII) (Fig. 1). The variations were of two types. The first was due to the loss or gain of a restriction site resulting in either the absence of two fragments that map adjacent to each other and the presence of a novel fragment equal in size to the sum of the two missing fragments, or in the loss of a single fragment and the appearence of two novel fragments the sum of whose sizes is equal to that of the missing fragment. This type of variation is indicative of some genetic heterogeneity between different isolates, as would be expected. The second type of variation observed was the difference in sizes of terminal fragments derived from different isolates, indicating variability in the size of the terminal inverted repeat. Such

TABLE 1. Details of patients with genital and non-genital lesions

Lesions	No. of samples	No. of patients		Patients' ages
		Males	Females	
Genital	48	7	41	11–63 years (mean 25·1 years)
Non-genital	45	19	26	2–70 years (mean 23 4 years)

TABLE 2. Numbers of MCVI and MCVII isolates in various groups of patients

	No. of patients		
	MCVI	MCVII	P
All patients	71	22	
Males	23	3	NS
Females	48	19	
Genital lesions	36	12	NS
Non-genital lesions	35	10	
Atopic dermatitis	7	1	NS
No atopic dermatitis	64	21	
< 15 years old	15	0	$<0{\cdot}05$
15 years or over	56	22	
Non-genital lesions			
< 15 years old	14	0	$<0{\cdot}05$
15 years or over	21	10	

variations were symmetrical at both ends of the viral genome as has been observed for orthopoxviruses, where it was taken as evidence of transient circularization during viral replication.[12]

Forty eight isolates were from genital lesions and 45 from non-genital lesions. In the genital lesions the ratio of MCVI:MCVII was 36:12 (3:1) and this was not significantly different from that in non-genital lesions, 35:10.

In patients under 15 years old, the MCVI:MCVII ratio was 15:0. This was significantly different from the ratio of 56:22 (2·55:1) found in older patients ($P < 0{\cdot}05$). Removal of the one child with genital lesions, a boy aged 11 years, allowed comparison of non-genital lesions in children and adults and again there was a significant difference. In those under 15 years the MCVI:MCVII ratio was 14:0. In those 15 years and older the ratio was 21:10 ($P < 0{\cdot}05$)

No consistent morphological differences were seen between MCVI and MCVII lesions.

DISCUSSION

Darai *et al.*[8] identified two MCV DNA patterns, MCVI and MCVII, as did Porter and Archard.[11] Earlier, Parr *et al.*[7] had described three MCV DNA fragment patterns, designated types I, II and III. Their type I corresponds to the MCVII of Darai *et al.*[8] and Porter and Archard.[11] The Parr types II and III were similar and appear to correspond to the MCVI of Darai *et al.* and Porter and Archard.[8,9,11]

Amongst the 10 isolates reported by Parr *et al.*[7] there were six type I, one type II and three type III. There were two penile lesions—both yielding Parr type I, which is equivalent to MCVII. The 14 isolates reported by Darai *et al.*[8] included only one MCVII and this was obtained from a vaginal lesion. In a subsequent report[9] this series had enlarged to 40 isolates and all but one were MCVI. In the combined series of 50 there were only seven MCVII isolates (ratio of MCVI:MCVII 7·29:1) and MCVII was the only type found in the three documented genital lesions.

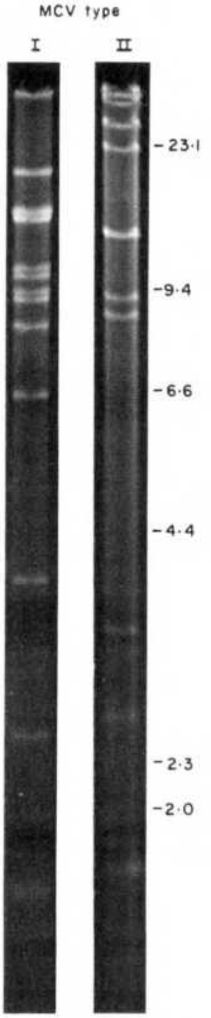

FIGURE 1. Representative BamHI endonuclease cleavage patterns of MCVI and MCVII DNA. The numbers on the right refer to molecular weight markers (kilobase pairs).

It thus appeared from these earlier studies that MCVII occurred comparatively infrequently and might be prefentially found at genital sites. In our series of 93 isolates we have confirmed that MCVII occurs less frequently than MCVI, the ratio of MCVI to MCVII being 3·23 : 1. MCVII was just as likely to be found in non-genital as in genital lesions and there was no indication that MCVII predominated at genital sites.

A statistically significant finding was that no patient under 15 years old had MCVII in a lesion at any site. The reasons for this are a matter for speculation.

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REFERENCES

1 Jablonska S, Orth G. Human Papoviruses. In: *Recent Advances in Dermatology* 6 (Rook AJ, Maibach HI, eds) Edinburgh: Churchill Livingstone, 1983; 1–36.

2 Orth G, Favre M. Human papillomaviruses: biochemical and biologic properties. *Clinics in Dermatology* 1985; **3:** 27–42.

3 Chaney SMJ, Warren KG, Kettyls J *et al.* A comparative analysis of restriction enzyme digests of the DNA of herpes simplex virus isolated from genital and facial lesions. *J Gen Virol* 1983; **64:** 357–71.

4 Corey L, Whitley RJ, Stone EF, Mohan K. Differences between herpes simplex virus type 1 and type 2 neonatal encephalitis in neurological outcome. *Lancet* 1988; **i:** 1–4.

5 Oriel JD. The increase in molluscum contagiosum. *Br Med J* 1987; **294:** 74.

6 Becker TM, Blount JH, Douglas J, Judson FN. Trends in molluscum contagiosum in the United States 1966–1983. *Sex Trans Dis* 1983; **13:** 88–92.

7 Parr RP, Burnet JW, Garon CF. Structural characterisation of the molluscum contagiosum virus genome. *Virology* 1977; **81:** 247–56.

8 Darai G, Reisner H, Scholz J *et al.* Analysis of the genome of Molluscum contagiosum virus by restriction endonuclease analysis and molecular cloning. *J Med Virol* 1986; **18:** 29–39.

9 Reisner H, Scholz J, Darai G. Characterisation of the genome of molluscum contagiosum; detection of molluscum contagiosum virus type 2. *Abstracts of Papers presented at the 6th Workshop on Poxvirus/Iridovirus.* Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 1986; 17.

10 Porter CD, Muhlemann MF, Cream JJ, Archard LC. Molluscum contagiosum: characterisation of viral DNA and clinical features. *Epidem Inf* 1987; **99:** 563–7.

11 Porter CD, Archard LC. Characterisation and physical mapping of molluscum contagiosum virus DNA and location of a sequence capable of encoding a conserved domain of epidermal growth factor. *J Gen Virol* 1987; **68:** 673–82.

12 Dumbell KR, Archard L. Comparison of white pock(h) mutants of monkeypox virus with parental monkeypox and with variola-like viruses isolated from animals. *Nature* 1980; **286:** 29–32.

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Free fatty acids and sterols in human eccrine sweat

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SUMMARY

The lipid content of human sweat was determined in thermally induced sweat collected over a Vaseline® or silicone barrier placed on the skin (clean sweat) and in sweat scraped from the skin surface without a barrier coating (scraped sweat). Lipids were extracted from concentrated sweat samples into chloroform:methanol and estimated by thin-layer chromatography in conjunction with photodensitometry. Scraped sweat contained 4 to 10 times more lipid than clean sweat and included cholesterol sulphate and ceramides resembling those found in the stratum corneum. In contrast, clean sweat contained only small amounts of free fatty acids and sterol. A marked individual and daily variation in sweat lipid content was also noted. The study indicates the importance of avoiding epidermal contamination when collecting sweat and the usefulness of our sweat collection method.

Perhaps the most common functional change occurring in the skin is dryness, or asteatosis, which may result from excessive evaporation of water from the stratum corneum.[1] Although dry skin occurs less frequently during warm weather, the role of eccrine sweating in preventing dry skin is unclear. It is not known whether eccrine sweating alleviates dry skin by simply hydrating the stratum corneum, or whether eccrine sweat contains lipids which lubricate the skin surface. Cytochemical studies have indicated that eccrine secretory cells (both clear and dark cells) contain lipid inclusion bodies[2] with an enzyme presumed to be a phospholipase at the periphery of the droplets.[3] Peter *et al.*[4] reported that thermally-induced human eccrine sweat contains large amounts of both free and ester-linked fatty acids,[4] and the odorous substance trans-3-methyl-2-hexenoic acid has been found in the sweat of schizophrenic patients.[5,6] Unfortunately, these studies were all done with sweat that was probably contaminated with both sebaceous and epidermal lipids. Recently, we have developed a simple method of sweat collection that can effectively minimize such contamination.[7,8] The purpose of the present study was to determine whether lipid is present in human sweat using our new sweat collection method which minimizes contamination by epidermal and sebaceous lipid.

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METHODS

Subjects
Five healthy men, aged 18–25 years, took part in the study.

Collection of sweat
Clean sweat. Thermally induced sweat was collected as described by Boysen *et al.*[7] with minor modifications.[8] Briefly, the skin on the upper trunk was gently cleansed with 0·5% Triton X, rinsed thoroughly with distilled water, and air-dried. A sheet of polyethylene film, 30 × 45 cm, was secured along its borders, to the subject's back, using a fast curing epoxy glue (Tra-Bond 2601 T, Tra-Con, Inc., Medford, MA, U.S.A.) in such a way as to create a storage pouch for sweat collection at its lower border. The skin inside the sweat collector (25 × 35 cm) was painted with a thin layer of high viscosity white petrolatum (Vaseline®) or silicone grease (Dow Corning), but unlike the original method,[7] the inside of the sweat collector was not filled with mineral oil. The plastic film was thus allowed to stay in loose contact with the Vaseline or silicone coated skin. The subject then entered an environmental room at 50°C and 70% relative humdity for 30 min. Sweat collected in the pouch was sampled every 5 min through tubing connected to a syringe, pooled and stored immediately on ice. The maximum sweat rate ranged from 20 to 35 $ml/min/m^2$, which was consistent with that found in our previous study.[7]

During profuse sweating in the environmental room, sweat ran rapidly over the Vaseline or silicone coated skin, and therefore, the length of time freshly secreted sweat was exposed to Vaseline or silicone may have been less than 5 min. We have already shown that contact with Vaseline does not alter the concentrations of several non-lipid sweat components.[7,8] In two preliminary experiments, we studied the extent to which lipids were extracted by Vaseline or silicone during a 5-min period. In these experiments, 100 ml of an aqueous solution containing 5 μg of cholesterol and 5 μg of palmitic acid were vigorously stirred in a 250 ml beaker the inside wall of which had been painted with silicone or Vaseline. Samples were then processed as described for sweat. The mean recovery of palmitic acid was 89·0%, and that of cholesterol 98·5%.

Scraped sweat. Scraped sweat was obtained by having the subject gently scrape beads of sweat from the chest (previously cleansed as described for the back) with a glass beaker. The evaporative water loss from scraped sweat during collection has been estimated to be about 30%.[7] This was corrected for by adding distilled water ($\frac{1}{3}$ of the sample volume) to each sample.

Lipid analyses The mean volume (± SD) of individual samples was 144 ± 32 ml, and one pooled sample from five individuals was 600 ml. The first few individual samples were concentrated to approximately 3 ml by ultrafiltration. In order to eliminate the possibility that lipids were being lost by adsorption onto the ultrafiltration membrane, subsequent samples were lyophilized and reconstituted in 3 ml distilled water. No differences were found in the results obtained with the two concentration methods.

The concentrated sweat samples were extracted twice with an equal volume of chloroform. The two chloroform extracts were combined and the chloroform evaporated under a gentle stream of nitrogen. The lipid residue was redissolved in 50 μl of chloroform:methanol (2:1).

Thin-layer chromatography (TLC) was performed on 0·25 mm thick layers of silica gel G (E. Merck, Darmstadt, West Germany) scored into 6-mm-wide lanes. Samples and standards were applied 2 cm from the bottom of the 20 × 20 cm plate. In preliminary experiments, the plates were developed to 10 cm with chloroform:methanol:water, 40:10:1, followed by two developments to the top with chloroform:methanol:acetic acid, 190:9:1. This procedure resolved cholesterol sulphate, ceramides and non-polar lipids, but cholesterol and fatty acids were not separated from one another. If phospholipids had been present they would have been moved from the origin and separated from the other lipids by this development. The small amount of material remaining at the origin was thought to be protein. Since these preliminary experiments indicated that ceramides and cholesterol sulphate were not present in the clean sweat, a less polar solvent system was adopted to separate the nonpolar lipids which were present in clean sweat. In the later experiments on clean sweat, plates were developed to the top with hexane:ethyl ether:acetic acid, 70:30:1, to resolve the fatty acids and sterols. Previously prepared total epidermal lipids, isolated pig epidermal ceramides[9,10] and commercially available cholesterol and palmitic acid (Sigma Chemical Co., St Louis, MO, U.S.A.) were used as standards.

After development, TLC plates were sprayed with 50% aqueous sulphuric acid and charred by slowly heating to 220°C. After cooling, the charred chromatograms were measured quantitatively by photodensitometry.[11]

RESULTS

In preliminary experiments, it was found that the total lipid content of scraped sweat samples was 0·3 to 1·0 μg/ml, which was 4–10 times more than in clean sweat samples. As illustrated in Figure 1a, the lipids from scraped sweat resembled those found in the stratum corneum[12,13] in that they included cholesterol sulphate, a series of ceramides, and cholesterol + fatty acids. In contrast, clean sweat samples (Fig. 1b) contained only one significant lipid band, which corresponded to cholesterol + fatty acids. Ceramides are completely absent from clean sweat, and only a trace of what was probably cholesterol sulphate was present.

Further experiments were carried out to resolve and quantitate free fatty acids and sterols present in clean sweat samples. The separation of fatty acids from sterols is illustrated in Figure 1c, and the quantitative results are sumarized in Table 1.

The results presented in Table 1 indicate some individual variation in the concentrations of fatty acids and sterols in sweat. Fatty acid concentrations ranged from less than 0·01 to 0·1 μg/ml, while the sterol concentrations ranged from less than 0·01 to 0·12 μg/ml. Variation was also seen between samples collected from the same individual on separate occasions. The first sweat sample collected from Subject 1 contained the highest lipid concentration, and subsequent samples contained much less. Whether this is due to depletion of lipids in the sweat glands is not known.

DISCUSSION

We have shown that human sweat collected with minimal epidermal contamination (clean sweat) contains very small amounts of cholesterol and free fatty acids. Scraped sweat, after adjustment of the volume to compensate for evaporative water loss during collection, contained 4 to 10 times more lipid that clean sweat. It appears that sweat samples can be massively containinated with lipids of sebaceous or epidermal origin if simply collected from the skin surface as in previous studies. The use of a barrier ointment (Vaseline or silicone) minimizes such contamination. Also, it is important to note that lipids do not partition significantly from the aqueous phase into the barrier layer under the conditions described here. In the present study, the fatty acid concentration found in scraped sweat was similar to that previously

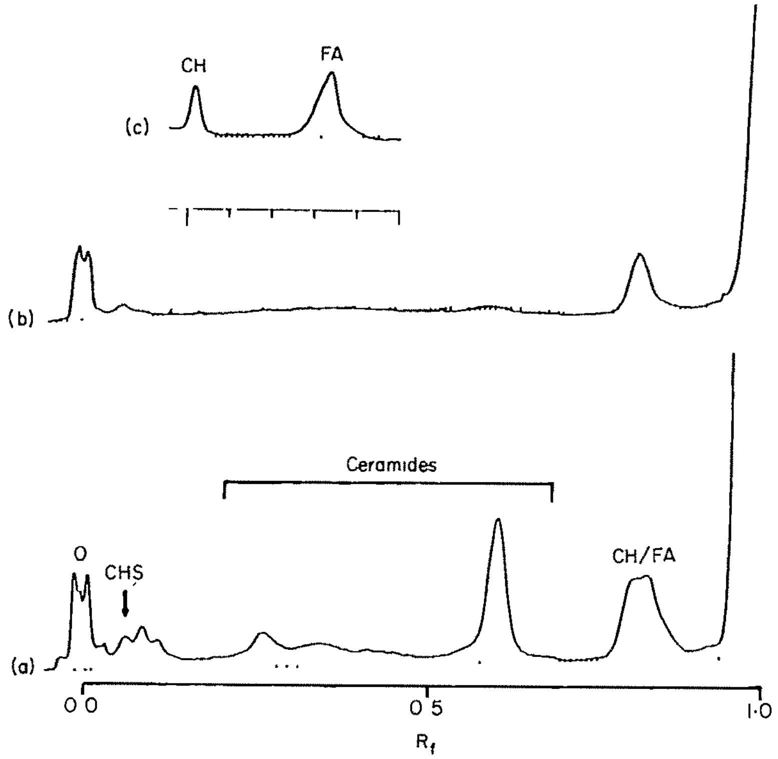

FIGURE 1. Photodensitometer profiles of the lipids from (a) scraped and (b) clean sweat. Insert (c) is part of a densitometer tracing illustrating the separation of fatty acids from sterols. O = origin; CHS = cholesterol sulphate; CH/FA = unresolved cholesterol + fatty acids; CH = cholesterol, FA = fatty acids.

TABLE 1. Amounts of free fatty acid and sterol in clean sweat samples

Subject no.	Barrier	Sterol (μg/ml)	Fatty acid (μg/ml)
1^{1}*	Vaseline	0·05	0·05
1^{2}	Silicone	< 0·01	< 0·01
1^{3}	Vaseline	< 0·01	0·01
2	Vaseline	0·12	0·10
3	Silicone	0·06	< 0·01
4	Vaseline	0·01	0·01
Five pooled samples	Vaseline	0·02	0·06
Mean (± SD)		0·04 (± 0·04)	0·04 (± 0·04)

* Subject 1 provided 3 sweat samples in a 1 week period.

reported by Peters *et al.*,[4] but the fatty acid concentration in clean sweat was 10- to 100-fold lower. Furthermore, scraped sweat, but not clean sweat, contained cholesterol sulphate and ceramides, which can be derived from the stratum corneum. These observations support the suggestion that the sterol and free fatty acids found in clean sweat may be products of the sweat gland (including the lipid derived from the secretory portion and possibly also from the duct) rather than epidermal contaminants. This is not surprising in view of the finding of lipid droplets and phospholipase-like enzymes at the periphery of both clear and dark cells.[2,3] Our results have also shown that there is individual and daily variation in the lipid content of sweat. Overall, this study has indicated that sweat can be easily contaminated by epidermal or sebaceous lipids unless precautions are taken to minimize this.

ACKNOWLEDGMENTS

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REFERENCES

1 Fitzpatrick TB, Bernard J. In: *Dermatology in General Medicine.* (Fitzpatrick TB, Eizen AZ, Wolff K, eds) 3rd edition, New York: McGraw Hill, 1987; 20–68
2 Spicer SS, Prioleau WH Jr. Ultrastructure of lipid inclusions and dense bodies in the human sweat gland. *Lab Invest* 1972; **27**: 7–16.
3 Spicer SS, Prioleau WH Jr. Cytochemical studies of lipid and acid phosphatase in the human eccrine sweat gland. *Lab Invest* 1972; **27**: 7–16.
4 Peter G, Schroepl F, Feisel HG, Thuerauf W. Gaschromatographische Untersuchungen von freien und gebundenen Fettsauren im ekkrinen Schweiss. *Arch Klin Exp Dermatol* 1970; **238**: 154–9.
5 Smith K, Thompson GF, Koster HD. Sweat in schizophrenic patients: Identification of odorous substance. *Science* 1969; **166**: 398–9.
6 Smith RV, Leong A. Methods for the preparation of trans-3-methyl-2-hexenoic acid, the malodorous component of schizophrenics' sweat. *J. Pharmaceutical Sci* 1972; **61**: 316–17.
7 Boysen TC, Yanagawa S, Sato F, Sato K. A modified anaerobic method of sweat collection. *J Appl Physiol* 1984 **56**: 1302–7.
8 Yokozeki H, Sato K. 350 kD thiol-dependent aminopeptidase as a marker of epidermal contamination in sweat. *J Appl Physiol* 1987; **63**: 1040–1048.
9 Wertz PW, Downing DT. Acylglucosylceramides of pig epidermis: structure determination. *J Lipid Res* 1983; **24**: 753–8.
10 Wertz PW, Downing DT. Ceramides of pig epidermis: structure determination. *J Lipid Res* 1983; **24**: 759–65.
11 Downing DT. Photodensitometry in thin-layer chromatographic analysis of neutral lipids. *J Chromatogr* 1968; **38**: 91–9.
12 Yardley HJ, Summerly R. Lipid composition and metabolism in normal and diseased epidermis. *Pharmacol Ther* 1981; **13**: 357–83.
13 Long SA, Wertz PW, Strauss JS, Downing DT. Human stratum corneum polar lipids and desquamation. *Arch Dermatol Res* 1985; **277**: 284–7.

British Journal of Dermatology (1989) **120**, 49–58.

An enzymatic method for distinguishing the stereoisomers of 12-hydroxyeicosatetraenoic acid in human epidermis and psoriatic scale

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SUMMARY

Homogenates of normal human epidermis synthesized 12-hydroxyeicosatetraenoic acid (12-HETE) when incubated *in vitro* with arachidonic acid. The stereoconfigurations of the C-12 hydroxyl isomers were determined by incubation with potato 5-lipoxygenase. The synthesized substrate-specific diHETEs; 5S, 12R and 5S, 12S, were readily separated by high performance liquid chromatograpy. Using this novel methodology, the normal epidermis was found to synthesize predominantly 12-S-HETE while, in contrast, psoriatic scale was found to contain 12-R-HETE. The 5-lipoxygenase inhibitors, Merck L-651, 896, Takeda AA861, and the active metabolite of Syntex lonapalene were found to inhibit 12-HETE formation in normal epidermal homogenates.

Archidonic acid (AA) was first shown to be converted to 12-hydroxyeicosatetraenoic acid (12-HETE) in human platelets.[1] The discovery of the presence of significant quantities of 12-HETE in psoriatic human epidermis[2] has led to a decade of experimentation to decipher its role in the pathology of this skin disease. 12-HETE is chemotactic for neutrophils,[3] stimulates epidermal cell proliferation[4,5] and induces histological changes characteristic of inflammatory skin diseases.[6–8]

There is recent evidence that psoriatic scale contains 12-R-HETE, and not 12-HETE which is formed in platelets.[9] The pharmacological modulation of 12-HETE synthesis also differs depending upon the cellular source of the enzyme. Nakadate *et al.*[10] showed that the Takeda compound AA861 inhibited mouse epidermal 12-lipoxygenation, but did not inhibit bovine platelet 12-lipoxygenase product formation. Neither AA861 nor an active metabolite of lonapalene inhibited human platelet derived 12-lipoxygenase, but did inhibit human epidermal 12-lipoxygenase.[11] Together, these studies suggest the existence of two distinct 12-lipoxygenases. With dermatological disorders as targets for therapy, the evaluation of 12-lipoxygenase

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inhibitors must utilize the skin as the source of enzyme, and we have done this in the present study. In addition, we present novel enzyme-based methodology for the identification of C-12 hydroxyl stereoisomers of arachidonic acid in which potato 5-lipoxygenase is incubated with 12-HETEs to yield reaction products which are readily resolved by reversed phase-high performance liquid chromatography. This technique provides an alternative method to the chemical derivatization used previously.[9]

METHODS

Reagents

Dulbecco's phosphate buffered saline (PBS) was purchased from Gibco Laboratories, Grand Island, NY, U.S.A. [1-^{14}C]-arachidonic acid (55 mCi/mmol) and 12-L-[5,6,8,9,11,12,14,15-^{3}H(N)]-HETE (225 Ci/mmol) were obtained from New England Nuclear, Boston, MA, U.S.A. Trypsin (porcine pancreatic type II) and nordihydroguaiaretic acid were from Sigma Chemical Co., St. Louis, MO, U.S.A. 12-S-HETE, 12-R-HETE, 5(S),12(S)-dihydroxy-6-trans,8-cis,10-trans,15-cis-eicosatetraenoic acid and 5(S),12(S)-dihydroxy-6-trans,8-cis,10-trans,14-cis-eicosatetraenoic acid and 5(S), 12(R)-dihydroxy-6-trans,8-cis,10-trans,14-cis-eicosatetraenoic acid were prepared and provided by Dr Brian Fitzsimons, Merck Frosst Laboratories, Quebec, Canada. 12-hydroxyheptadecatrienoic acid (HHT) was purchased from Biomol Research Laboratories, Inc., Philadelphia, PA, U.S.A. The compound 2-diethylaminoethyl-2,2-diphenylvalerate hydrochloride (SKF525A) was obtained from Smith, Klein and French Laboratories, Philadelphia, PA, U.S.A. The compounds 2,3-di-hydro-6-[3-(2-hydroxymethyl)phenyl-2-propenyl]-5-benzofuranol (L-651,896),2-(12-hydroxydodeca-5,10-diynyl)-3,5,6-trimethyl-1,4-benzoquinone (Takeda AA861) and 6-chloro-2,3-dimethoxy-1,4-naphthalenedione (the active metabolite of Syntex lonapalene) were synthesized in our laboratories. All other chemicals were reagent grade.

Skin specimens

Biopsies of normal skin were obtained either from patients undergoing surgical procedures or from fresh cadavers, and were stored at −70°C. Psoriatic scale was collected from three patients with chronic psoriasis vulgaris and stored at −20°C.

Preparation of epidermal homogenates

Full-thickness skin samples were thawed and subcutaneous fat was removed and discarded. Epidermal tissue was separated from dermal tissue by the method described by Kragballe *et al.*[12] or by incubation with 1% trypsin in PBS for 30 min at 55°C. Epidermal strips were frozen in liquid nitrogen and pulverized in a Spex Freezer Mill (Spex Industries, Inc., Edison, NJ, U.S.A.). The powdered epidermis was suspended in PBS (pH 7·4) containing 2 mM dextrose and 3 mM reduced glutathione (1 ml/100 mg tissue). The suspension was then sonicated with three 10 s pulses in a Braun-Sonic 2000 sonicator (at 350 W) at 4°C and centrifuged at 1500 *g* for 10 min at 4°C. In some experiments, the 1500 *g* supernatant was further centrifuged at 105,000 *g* for 60 min. The resulting supernatant represented a cytosolic preparation and the resuspended pellet represented the microsomal fraction of the cells.

Incubations and extractions

Epidermal homogenates (0·5 ml of 1500 *g* supernatant) were incubated with 18·4 μM ^{14}C-AA at 37°C. When evaluations of compounds were performed, the enzyme preparations were incubated with the compounds for 5 min at 37°C prior to addition of substrate (final ethanol concentration 1·4%). The incubations were terminated by the addition of 2 ml ice-cold methanol and after 15 min on ice the samples were centrifuged at 100 *g* for 10 min. The supernatant was diluted with deionized water to 15% methanol. The samples were then concentrated on prewashed C-18 Sep-Pak cartridges (Waters Associates, Milford, MA, U.S.A.). Eicosanoids were eluted with methanol, which was then evaporated under N_2, and resuspended in 30% methanol prior to HPLC. Using [^{3}H]-12-HETE, the mean recovery efficiency ($\pm$ SD) by this method was found to be $84 \pm 11\%$ ($n = 6$).

High performance liquid chromatography

Eicosanoids were separated by reversed-phase HPLC (25 cm Supelcosil C-18 column, 5 μm particle, Supelco, Inc., Bellefonte, PA, U.S.A.) with absorbances and spectra measured by a HP-1040A Spectrophotometric detector (Hewlett Packard, Parmus, NJ, U.S.A.). The isocratic elution conditions were: 73% methanol, 27% water, 0·05% glacial acetic acid, 0·5 mM oxalic acid, pH 5·6, flow rate 1 ml/min, at 34°C. Authentic internal standards were used during each run with radiolabelled samples. Appropriate fractions were collected and radioactivity was measured in a model 2000 Ca Tri-Carb liquid scintillation analyser (Packard Instrument Company, Sterling, VA, U.S.A.).

Protein analysis

Protein content of the enzyme preparations was determined by the method of Lowry *et al.*[13]

Potato 5-lipoxygenase reaction with 12-R/S-HETE

A crude source of 5-lipoxygenase was prepared from potatoes using a modification of the method of Sekiya *et al.*[14] Briefly, a Russet potato tuber was homogenized for 3 min at 4°C using a Sorvall Omni-Mixer in a buffer (1 g/1·6 ml) containing 0·1 M sodium acetate, 2 mM ascorbic acid, 2 mM metabisulphite, pH 4·5. The homogenate was filtered through cheese cloth and then centrifuged at 15,000 *g* for 20 min at 4°C. The supernatant was passed through three prewashed C-18 Sep-Pak columns in tandem to remove excess endogenous lipids. This enzyme preparation (0·1 ml) was incubated with 12-HETE (in 0·01 ml methanol) after addition of 0·9 ml 0·1 M potassium phosphate, pH 6·5 (final pH 6·2). After 30 min at 25°C the reaction was terminated by loading the sample onto a C-18 Sep-Pak column. The products of the reaction were eluted from the column with methanol, and after evaporation under N_2, were resuspended in 30% methanol and analysed by HPLC under the same conditions described for eicosanoid separations.

RESULTS

Arachidonic acid product formation by normal epidermal homogenates

Time-dependent synthesis of 12-HETE, 15-HETE and the cyclooxygenase product HHT was demonstrated in both the 1500 *g* supernatant (Fig. 1) and the 105,000 *g* pellet (data not shown). In most preparations, only 15-lipoxygenase activity was detected in the cytosol fraction (Fig. 2; data for 12-lipoxygenase not shown).

To test the possiblity that the cytochrome P_{450} system gave rise to the measured increases in 12-HETE, the microsomal fraction from the epidermal homogenates was isolated and incubated in the presence and absence of a constituent of the P_{450} system, NADPH. In addition, the inhibitor SKF525A was also evaluated for its effect on 12-HETE synthesis (Table 1). NADPH did not augment nor did SKF525A substantially diminish synthesis. It is concluded

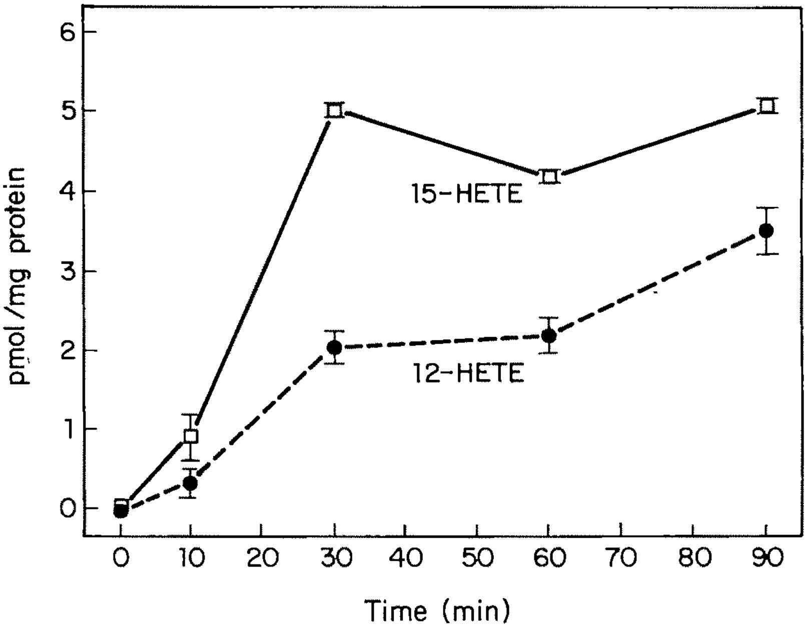

FIGURE 1. Time course of [^{14}C]15-HETE and [^{14}C]12-HETE formation by 1500 *g* supernatant from normal human epidermal homogenates. Results of a representative experiment are shown. Points are means and ranges of duplicate determinations.

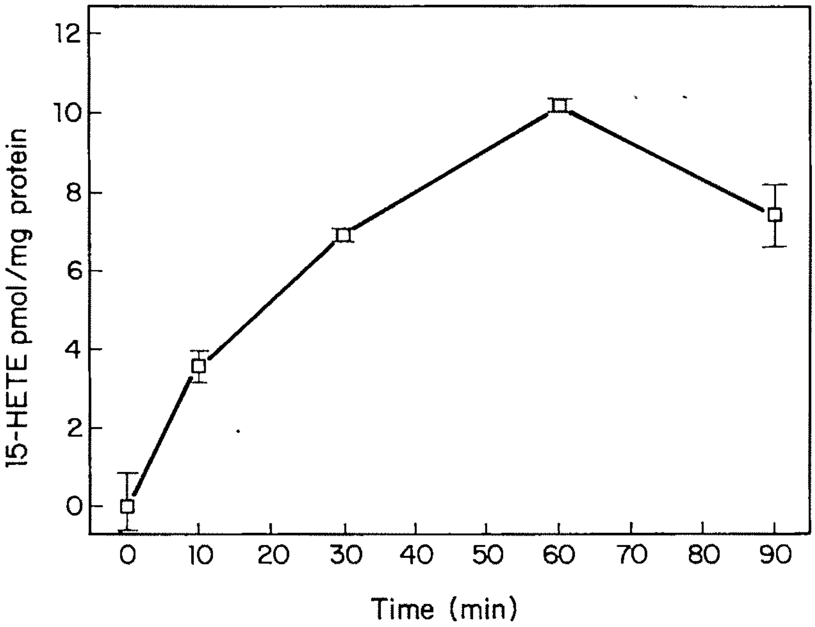

FIGURE 2. Time course of [^{14}C]15-HETE formation by 105,000 *g* supernatant from normal human epidermal homogenates. Results of a representative experiment are shown. Points are means and ranges of duplicate determinations.

TABLE 1. Effect of NADPH and SKF525A on 12-HETE synthesis by microsomal fraction of normal epidermal homogenates

NADPH 100 μM	SKF525A 100 μM	^{14}C-12-HETE pmol/mg protein/60 min*	% Control
+	−	22·8 ± 5·1	113
+	+	18·2 ± 0·0	90
−	−	20·2 ± 0·1	100

105,000 *g* pellet from homogenates of normal epidermis was resuspended and incubated for 5 min, at 37°C with SKF525A and/or NADPH prior to addition of ^{14}C-AA.

* Boiled enzyme control values were subtracted prior to calculations. Values are means ± range of duplicate determinations.

that most of the 12-HETE is derived via a lipoxygenase reaction and not from the cytochrome P$_{450}$ system.

Identification of C-12 hydroxyl enantiomers in normal epidermis and psoriatic scale

When authentic 12-S-HETE or 12-R-HETE were incubated with potato 5-lipoxygenase preparation, substrate-specific diHETEs, i.e. 5S,12S-diHETE and 5S,12R-diHETE, respectively, were formed (Fig 3). The identity of these reaction products was inferred by their co-migration on HPLC with 5S,12S-diHETE and 5S,12R-diHETE standards. In addition, the spectra of the potato 5-lipoxygenase reaction products were identical to the diHETE standards. The second peak in the doublets produced by incubations with 12-S-HETE and 12 R-HETE (Fig. 3) also had a spectrum consistent with a diHETE and may represent an all-trans isomer of the reaction product.

In order to determine which stereoisomer of 12-HETE was being measured in the human epidermal samples, HPLC fractions of 12-HETE were collected and incubated with the potato enzyme. Two epidermal homogenates, one from a surgical and the other from a cadaver specimen were incubated in the presence or absence of exogenous AA. When incubated for 60 min at 37°C without exogenous AA, the predominant enantiomer was found to be 12-S-HETE in both surgical and cadaver speciments (Fig. 4). Exogenous ^{14}C-labelled or unlabelled AA increased 12-HETE synthesis, but no increase of 5S,12R-diHETE was evident on HPLC after incubation with potato 5-lipoxygenase. This again suggested that only 12-S-HETE synthesis occurred in these samples.

Organic extracts of scale from three psoriatic patients were evaluated in a similar manner. All three samples of scale gave a distinctly different HPLC profile from that obtained with normal epidermis (Fig. 4). The predominant product of scale 12-HETE reacted with potato 5-lipoxygenase was 5S,12R-diHETE. The formation of this product was attributed to 12-R-HETE being the substrate.

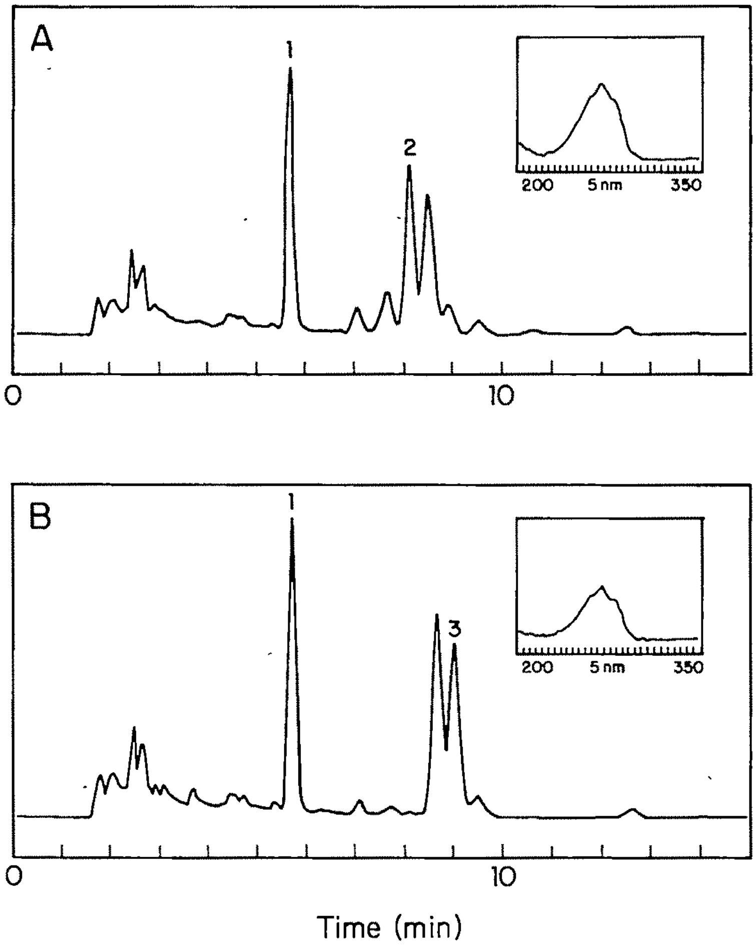

FIGURE 3. High performance liquid chromatograms (at 280 nm) of products from reaction of potato 5-lipoxygenase with A 12-S-HETE and B 12-R-HETE. 1: prostaglandin B_2 internal standard. 2: 5S, 12S-diHETE. 3: 5S, 12R-di-HETE. Spectra of respective diHETEs are shown as insets.

Pharmacological modulation of product formation in normal epidermal homogenates

The effect of inhibitory compounds on ^{14}C-AA metabolism was measured in the 1500 *g* supernatant (Table 2). At 10 μM, L-651,896 and the lonapalene metabolite inhibited 12- and 15-lipoxygenase activity, while AA861 and NDGA gave inhibition of lipoxygenase and cyclooxygenase activity. L-651,896 exhibited concentration-dependent inhibition of product formation (Table 3).

DISCUSSION

We have confirmed the findings of Woollard[9] that psoriatic scale contains 12-R-HETE. The epidermal homogenates *in vitro*, however, synthesized 12-S-HETE as the predominant

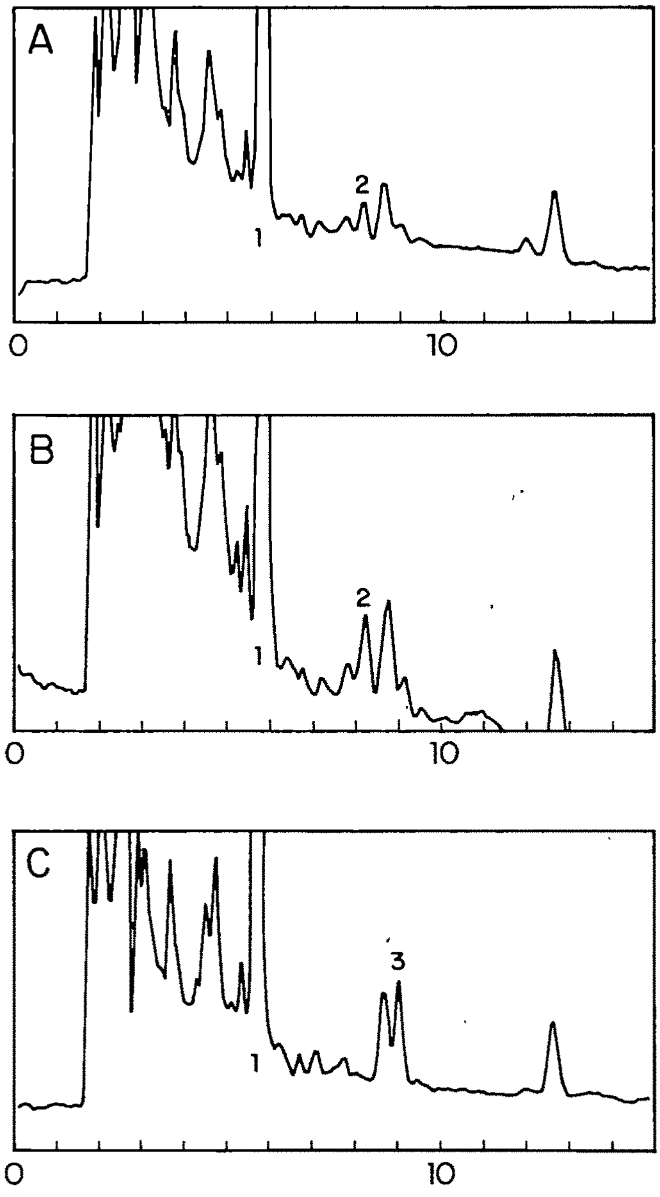

FIGURE 4. Representative high performance liquid chromatograms (at 280 nm) of products from reaction of potato 5-lipoxygenase with A epidermis-derived 12-HETE without exogenous AA, B epidermis-derived 12-HETE incubated with exogenous AA, and C psoriatic scale-derived 12-HETE. 1: prostaglandin B_2 internal standard. 2: 5S, 12S-diHETE. 3:5S, 12R-diHETE.

TABLE 2. Inhibition of eicosanoid synthesis in 1500 *g* supernatant from normal human epidermis

	% Inhibition		
	12-HETE	15-HETE	HHT
L-651,896 10 μM	98, 100	87, 64	36, 32
Lonapalene metabolite 10 μM	46, 78	55, 25	6, 16
AA861 10 μM	70, 99	77, 45	65, 52
NDGA 10 μM	100	99	100

Values are the means of duplicate determinations from two separate experiments.

TABLE 3. Effect of different concentrations of L-651,896 on eicosanoid synthesis in 1500 *g* supernatant from normal human epidermis

	15-HETE		12-HETE		HHT	
	pmol/mg protein*	% Inhibition	pmol/mg protein*	% Inhibition	pmol/mg protein*	% Inhibition
Control	39·3 ± 1·2	—	4·5 ± 0·5	—	4·3 ± 0·5	—
L-651,896 10 μM	2·5 ± 0·6	94	0·0 ± 0·1	100	2·4 ± 0·5	43
L-651,896 3 μM	10·7 ± 1·5	73	1·1 ± 0·1	74	3·7 ± 0·4	15
L-651,896 1 μM	27·3 ± 7·0	30	2·7 ± 0·3	39	3·5 ± 0·3	18

* Boiled enzyme control values subtracted prior to calculations. Values are means ± range of duplicate determinations.

enantiomer. This also confirms a recent report by Woollard and Leigh.[15] Greater biological activity of 12-R-HETE over that of 12-S-HETE has been demonstated in neutrophil chemotaxis[16] and its ability to aggregate rat leukocytes.[17] In addition, Evans *et al.*[17] showed that 12-S-HETE competitively displaces $[^3H]LTB_4$ binding to human leukocyte membranes and is at least 10-fold more potent than 12-S-HETE in displacing the radiolabelled ligand. If 12-R-HETE synthesis becomes activated during psoriasis, it could manifest these same activities indicative of the associated inflammation. Further understanding of the pathway may be provided by characterizing the 12-HETE enantiomer formed *in vitro* by lesional and non-lesional psoriatic epidermal preparations.

The 12-HETE measured in human epidermal homogenates does not appear to be a product of the cytochrome P_{450} system. This is consistent with the studies of Nakadate *et al.*[18] using mouse epidermal homogenates which showed that 12-HETE formation was not affected by carbon monoxide, an inhibitor of P_{450}-catalysed oxygenation reactions. Microsomal cytochrome P_{450} may also be excluded as the source of 12-HETE in our preparations, as 12-HETE was found to be the stereoisomer synthesized and the production of predominantly 12-R-HETE has been shown to be associated with P_{450} of rat liver.[19]

L-651,896 and lonapalene have been shown to be potent inhibitors of cell-free preparations of 5-lipoxygenase from rat basophilic leukaemia cells with IC_{50} values of 0·1 μM and 0·5 μM, respectively.[20,21] AA861 inhibited 5-lipoxygenase in the cytosolic fraction of guinea-pig peritoneal leukocytes with an IC_{50} of 0·8 μM.[22] Our results demonstrate that these compounds also have the ability to inhibit human epidermal lipoxygenases. L-651,896 can also inhibit platelet cytosol 12-lipoxygenase, but neither lonapalene nor AA861 have demonstrated that ability.[11,20,22] Both L-651,896 and lonapalene have been developed as topical therapeutic agents for psoriasis and thus it is appropriate to use a skin-derived rather than a platelet-derived system to evaluate these compounds for specificity of lipoxygenase inhibition.

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REFERENCES

1 Hamberg M, Samuelsson B. Prostaglandin endoperoxides. Novel transformations of arachidonic acid in human platelets. *Proc Natl Acad Sci USA* 1974; **71:** 3400–4.

2 Hammarstrom S, Hamberg M, Samuelsson B *et al.* Increased concentrations of nonesterified arachidonic acid, 12L-hydroxy-5,8,10,14-eicosatetraenoic acid, prostaglandin E_2, and prostaglandin $F_{2\alpha}$ in epidermis of psoriasis. *Proc Natl Acad Sci USA* 1975; **72:** 5130–4.

3 Goetzl EJ, Sun FF. Generation of unique mono-hydroxyeicosatetraenoic acids from arachidonic acid by human neutrophils. *J Exp Med* 1979; **150:** 406–11.

4 Chan CC, Duhamel L, Ford-Hutchinson AW. Leukotriene B_4 and 12-hydroxyeicosatetraenoic acid stimulate epidermal proliferation *in vivo* in the guinea pig. *J Invest Dermatol* 1985; **85:** 333–4.

5 Kragballe K, Fallon JD. Increased aggregation and arachidonic acid transformation by psoriatic platelets: evidence that platelet-derived 12-hydroxyeicosatetraenoic acid increases keratinocyte DNA synthesis *in vitro*. *Arch Dermatol Res* 1986; **278:** 449–53.

6 Dowd PM, Black AK, Woollard PM, *et al.* Cutaneous responses of 12-hydroxy-5,8,10,14-eicosatetraenoic acid. *J Invest Dermatol* 1985; **84:** 537–41.

7 Cunningham FM, Woollard PM, Camp RDR. Proinflammatory properties of unsaturated fatty acids and their monohydroxy metabolites. *Prostaglandins* 1985; **30:** 487–509.

8 Ruzicka T, Burg G. Effects of chronic intracutaneous administration of arachidonic acid and its metabolites. Induction of leukocytoclastic vasculitis by leukotriene B_4 and 12-hydroxyeicosatetraenoic acid and its prevention by prostaglandin E_2. *J Invest Dermatol* 1987; **88:** 120–3.

9 Woollard PM. Stereochemical difference between 12-hydroxy-5,8,10,14-eicosatetraenoic acid in platelets and psoriatic lesions. *Biochem Biophys Res Commun* 1986; **136:** 169–76.

10 Nakadate T, Yamamoto S, Aizu E, Kato R. Inhibition of mouse epidermal 12-lipoxygenase by 2,3,4-trimethyl-6-(12-hydroxy-5,10-dodecadiynyl)-1,4-benzoquinone (AA861). *J Pharm Pharmol* 1985; **37:** 71–3.

11 Opas EE, Argenbright LW, Pacholok SG *et al.* Differential effects of inhibitors on 12-lipoxygenase derived from porcine, murine and human skin and human platelet cytosol. *Clin Res* 1987; **35:** 795 (abstract).

12 Kragballe K, Desjarlais L, Duell EA, Voorhees JJ. *In vitro* synthesis of 12-hydroxyeicosatetraenoic acid is increased in uninvolved psoriatic epidermis. *J Invest Dermatol* 1986; **87:** 47–52.

13 Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951; **193:** 265–75.

14 Sekiya J, Aoshima H, Kajiwasa T *et al.* Purification and some properies of potato tuber lipoxygenase and detection of linoleic acid radical in the enzyme reaction. *Agric Biol Chem* 1977; **41:** 827–35.

15 Woollard PM, Leigh I. Sterochemical analysis of 12-hydroxy-5,8,10,14-eicosatetraenoic acid (12-HETE) from adult human epidermal cells *in vitro*. *Br J Dermatol* 1987; **116:** 437 (abstract).

16 Cunningham FM, Woollard PM. 12(R)-hydroxy-5-8,10,14-eicosatetraenoic acid is a chemoattractant for human polymorphonuclear leucocytes *in vitro*. *Prostaglandins* 1987; **34:** 71–78.

17 Evans JF, Leblanc Y, Fitzsimmons BJ *et al.* Activation of leukocyte movement and displacement of [^{3}H] leukotriene B$_4$ from leukocyte membrane preparations by (12R)- and (12S)-hydroxyeicosatetraenoic acid. *Biochim Biophys Acta* 1987; **917:** 406–10.

18 Nakadate T, Aizu E, Yamamoto S, Kato R. Some properties of lipoxygenase activities in cytosol and microsomal fractions of mouse epidermal homogenate. *Prostag Leuk Med* 1986; **21:** 305–19.

19 Capdevila J, Yadagiri P, Manna S, Falck JR. Absolute configuration of the hydroxyeicosatetraenoic acids (HETEs) formed during catalytic oxygenation of arachidonic acid by microsomal cytochrome P$_{450}$. *Biochem Biophys Res Commun* 1986; **141:** 1007-11.

20 Bonney RJ, Davies P, Dougherty H *et al.* Biochemical and biological activites of 2,3-dihydro-6-[3-(2-hydroxymethyl)-phenyl-2-propenyl]-5-benzofuranol (L-651,896), a novel topical anti-inflammatory agent. *Biochem Pharmacol* 1987; **36:** 3885–91.

21 Jones GH, Venuti MC, Young JM *et al.* Topical nonsteroidal antipsoriatic agents. 1.1,2,3,4-tetraoxygenated napthalene derivatives. *J Med Chem* 1986; **29:** 1504–11.

22 Yoshimoto T, Yokoyama C, Ochi K *et al.* 2,3,5-Trimethyl-6-(12-hydroxy-5,-10-dodecadiynyl)-1,4-benzoquinone (AA861), a selective inhibitor of the 5-lipoxygenase reaction and the biosythesis of slow-reacting substance of anaphylaxis. *Biochim Biophys Acta* 1982; **713:** 470–3.

British Journal of Dermatology (1989) **120**, 59–70.

Binding of bullous pemphigoid and pemphigus vulgaris sera to SCaBER cell line in an ELISA

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SUMMARY

Specific antibodies present in the sera of patients with bullous pemphigoid or pemphigus vulgaris were detected in an enzyme-linked immunosorbent assay (ELISA) employing a squamous carcinoma cell line, SCaBER, as substrate. Bullous pemphigoid sera bound preferentially to permeabilized cells, suggesting that the antigens are largely intracellular. The assay may prove to be a useful addition to current methods of detecting circulating antibodies in these patients.

Pemphigoid and pemphigus are autoimmune blistering diseases in which antibodies to components of the epidermis are present. In bullous pemphigoid, patients develop large, tense blisters. Antibodies and complement are fixed to the basement membrane zone (BMZ) of the epidermis. Circulating antibodies directed against the BMZ are present in at least 70% of patients.[1] However, there does not appear to be a correlation between clinical disease and antibody titre in these patients.[1,2] Cicatricial pemphigoid (benign mucous membrane pemphigoid) differs from bullous pemphigoid in that the mucous membranes are involved and scarring is common. Antibodies and complement are fixed to the BMZ, but circulating antibodies are present in only 20% of these patients.[3]

Pemphigus is characterized by erosions of the skin and mucous membranes, and antibodies are fixed to the intercellular substance (ICS) of the epidermis. The major clinical forms of the disease are pemphigus vulgaris and pemphigus foliaceus. The majority of patients with pemphigus vulgaris have circulating antibodies, and some studies have shown a correlation between antibody titre and disease activity[2,4] although this is not a universal finding.[5] The lesions in pemphigus foliaceus are more superficial and circulating antibodies are also directed against the ICS.

Antibodies from pemphigus sera and 25% of bullous pemphigoid sera have been shown by indirect immunofluorescence (IIF) to bind to the cell lines SCaBER and COLO 16.[6] Pemphigus and bullous pemphigoid sera were also shown to bind to SCaBER cells in a

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radioimmunoassay.[7] We have designed an ELISA using the cell line SCaBER as substrate, to produce a quantifiable and sensitive assay system. The ELISA was used to study sera from patients with pemphigoid or pemphigus and the clinical value of the assay was assessed, particularly in comparison with IIF titres on skin sections.

METHODS

Patient sera

Sera were obtained from 30 patients with a clinical diagnosis of pemphigoid. The diagnosis was confirmed histologically and by direct immunofluorescence (DIF) for immunoglobulin or C3 fixed to the BMZ. Sera from 25 patients had circulating antibodies detectable by IIF on human skin sections. The titres of these sera were between 1:20 and 1:640. Sera from four patients with bullous pemphigoid and one patient with cicatricial pemphigoid (CP) had no circulating antibodies detectable by IIF.

Sera were also obtained from eight patients with pemphigus vulgaris and seven patients with pemphigus foliaceus. The diagnosis was confirmed by histology and by DIF. The antibody titres ranged from 1:40 to 1:1280 for 13 of the sera. Sera from two patients with pemphigus foliaceus had no circulating antibodies detectable by IIF on human skin sections.

Control sera were taken from 14 individuals with non-malignant skin conditions or unrelated inflammatory dermatoses. Patient or control sera which showed anti-nuclear binding were not included in the assay, as the high level of antibody binding produced false positives. All sera were stored at −20°C.

Cell culture

The SCaBER cell line was derived from a human squamous cell carcinoma of the bladder.[8] The cells were passaged every 3–4 days using 0·25% trypsin and 0·1% EDTA and were seeded at 1×10^6 cells per 80 cm^2 flask in Dulbecco's Modified Eagle's Medium supplemented with 10% heat-inactivated fetal calf serum and 20 mmol/l HEPES (complete medium).

ELISA

SCaBER cells were seeded at a density of 2×10^4 cells in 200 μl of complete medium per well, in tissue culture grade flat-well microtitre plates. The cells were incubated at 37°C for 3 days in a humidified atmosphere, until confluent. The cells were washed three times with phosphate-buffered saline (PBS) pH 7·2 0·05% Tween 20 (PBS/Tween). Twofold dilutions of sera in PBS containing 1% ovalbumin and 0·1% sodium azide were made sequentially across the microtitre plate, to give a final volume of 75 μl per well. The plates were incubated for 1 h at room temperature, then washed three times in PBS/Tween.

Horseradish peroxidase-conjugated rabbit anti-human IgG (γ-chain specific, Dako), at a 1:1000 dilution in PBS containing 1% ovalbumin, was added at 50 μl per well. Sodium azide was not included in this step as it inactivates peroxidase. The plates were incubated for 1 h at room temperature, then washed twice in PBS/Tween and twice in PBS. Ortho-phenylenediamine (1 mg/ml in citric acid/sodium phosphate buffer, pH 5·0) and 0·01% hydrogen peroxide was added at 50 μl per well. The chromogenic reaction was stopped after 10 min with 25 μl of 2 mol/l sulphuric acid, and the optical density (OD) of each well was read at a wavelength of 492 nm.

Modification of ELISA

SCaBER cells were permeabilized prior to the assay using the non-ionic detergent, saponin.[9]

Preliminary work showed similar IIF levels for individual patient or control sera with SCaBER cells permeabilized using saponin, distilled water or by air-drying. Permeabilization with saponin produced the most consistent results in the small wells of the microtitre plates. SCaBER cells were grown in microtitre plates as described above. The cells were washed twice with PBS/Tween, then incubated with 200 μl per well 0·1%. saponin in PBS, for 30 min at room temperature. The cells were washed three times with PBS/Tween before use in the ELISA.

IIF on SCaBER cells

SCaBER cells were seeded onto alcohol-flamed glass coverslips in a 24-well plate, at 4×10^4 cells per well. The cells were incubated for 3–5 days at 37°C until confluent. The cells were washed three times with PBS. Viable cells, or cells permeabilized with saponin and washed a further three times, were incubated with a 1:20 dilution of patient serum in PBS, for 30 min at room temperature. Specific binding of patient sera could be clearly distinguished from non-specific binding of control sera at a 1:20 dilution. The cells were washed for 30 min with PBS, followed by incubation for 30 min with a 1:30 dilution of FITC-conjugated sheep anti-human immunoglobulin IgG, IgA and IgM (Wellcome, fluorescein/protein molar ratio 3·5, antibody concentration 4·3 mg/ml). The cells were washed for a further 30 min with PBS and the coverslips were mounted in buffered glycerol pH 8·0. These were observed using a Nikon inverted microscope fitted with an epifluorescence attachment and filter block B. The level of fluorescence was scored on an arbitrary scale from 0 to 3, where 0 = no fluorescence, 1 = weak fluorescence, 2 = intermediate fluorescence and 3 = strong fluorescence.

RESULTS

Patient or control sera were diluted from 1:8 to 1:2048 in the ELISA. The 1:256 dilution of serum was shown to give optimum discrimination between binding of patient sera to the cells and either non-specific binding of a reference control serum, or non-specific binding of sera to microtitre plates without cells, and non-specific binding of patient serum to a cell line derived from a transitional cell carcinoma of the bladder, J82.[10] The mean OD value for duplicate serum samples at 1:256 dilution was taken as the endpoint of the assay, and the corresponding mean value for duplicate samples of a reference control serum was subtracted to give the 'binding index', as shown in Figure 1. Twofold dilutions of horseradish peroxidase-conjugated rabbit anti-human IgG from 1:250 to 1:8000 were tested in the assay and a dilution of 1:1000 was shown to give the optimum range of OD readings at 492 nm (0·2 to 1·0).

The mean OD value (± SD) for eight replicate samples of reference control serum with viable cells was 0·07 ± 0·03 and with permeabilized cells was 0·63 ± 0·03. High non-specific binding is inevitable in an ELISA in which unpurified antigens are used as substrate with whole serum. Background binding was reduced by the use of a non-specific protein (ovalbumin) and Tween 20 in the ELISA. These background values were subtracted from the OD values for the patient and control sera, to allow the specific binding to viable or permeabilized cells to be visualized more easily.

The mean binding index (± SD) and the intra-assay and inter-assay variation was assessed using eight replicate samples of a positive serum. The mean binding indices were 1·06 ± 0·05 (intra-assay coefficient of variation 4·74%) and 0·97 ± 0·04 (inter-assay coefficient of variation 4·41%, respectively.

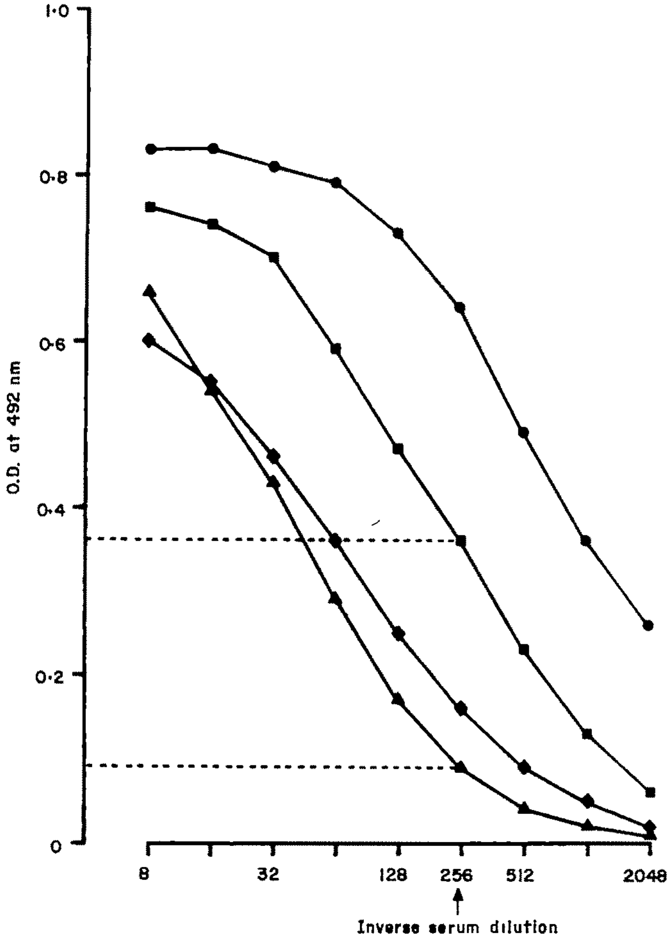

FIGURE 1. Binding of bullous pemphigoid (●), pemphigus vulgaris (■), pemphigus foliaceus (♦) or reference control serum (▲) to SCaBER cell line by ELISA. Each point represents the mean of duplicate samples. Binding index = mean OD for 1:256 dilution of test serum − mean OD for 1·256 dilution of reference control serum.

Pemphigoid sera

The binding of patient and of control sera to SCaBER cells in the ELISA is shown in Figure 2. The bullous pemphigoid sera which had titres of 1:20 or greater on skin sections (IIF-positive) showed significantly higher binding in the ELISA than control sera ($P < 0{\cdot}005$; Mann–Whitney *U*-test). One of the IIF-negative bullous pemphigoid sera gave binding above control levels in the assay, but binding in this group as a whole was not significantly different from the controls.

Binding of sera to intact cells was compared with 0·1% saponin permeabilized cells in the ELISA, and this is shown in Figure 3. The control sera showed significantly reduced binding to permeabilized cells compared with intact cells ($P < 0{\cdot}01$; Wilcoxon signed rank test), which was due to subtraction of high levels of background binding. When this high non-specific binding was subtracted from test sera, the binding index values appeared to be reduced. In contrast, the IIF-positive bullous pemphigoid sera showed significantly increased binding to the permeabi-

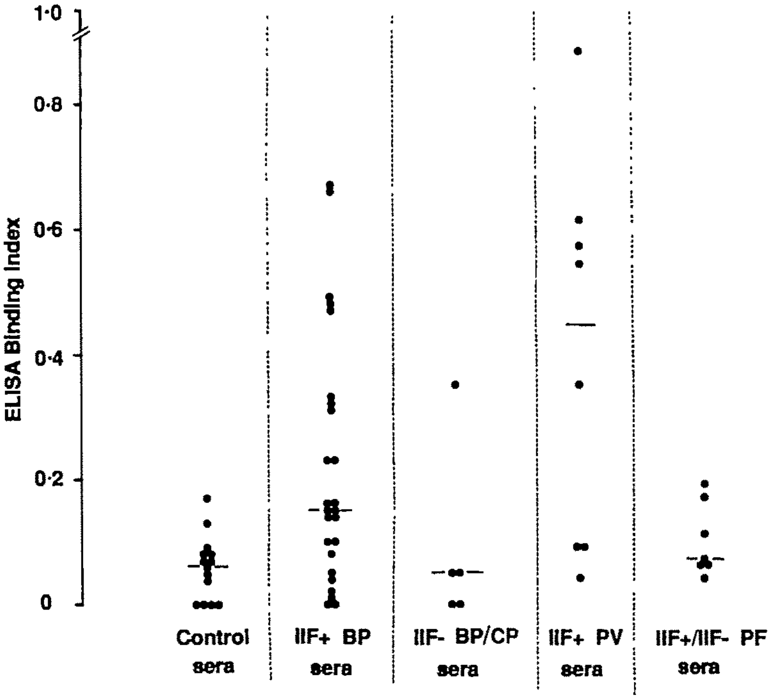

FIGURE 2. Binding of bullous pemphigoid (BP), cicatricial pemphigoid (CP), pemphigus vulgaris (PV), pemphigus foliaceus (PF) and control sera to the SCaBER cell line, by ELISA. Sera were grouped according to the presence (IIF +) or absence (IIF −) of antibodies by indirect immunofluorescence on skin sections. Bars indicate medians. IIF-positive bullous pemphigoid sera and IIF-positive pemphigus vulgaris sera showed significantly more binding in the ELISA, than control sera ($P < 0{\cdot}005$).

lized cells ($P < 0{\cdot}001$ Wilcoxon signed rank test). The IIF-negative bullous pemphigoid and chronic pemphigoid sera showed reduced binding compared with intact cells, as seen with the control sera. Figure 4 shows the differences in binding of individual sera to intact and to permeabilized cells. All, but four, of the IIF-positive bullous pemphigoid sera showed greater binding to the permeabilized cells. All control sera showed reduced binding to permeabilized cells.

Pemphigus sera

The binding of patient and control sera to SCaBER cells in the ELISA is shown in Figure 2. Five of the eight IIF-positive pemphigus vulgaris sera showed significant binding in the ELISA compared with the control sera ($P < 0{\cdot}005$; Mann–Whitney U-test). Pemphigus foliaceus sera did not bind above control levels in the assay.

Binding of pemphigus sera to intact and to permeabilized cells by ELISA is shown in Figure 3. The IIF-positive pemphigus vulgaris and pemphigus foliaceus sera did not show significant differences in binding to intact and to permeabilized SCaBER cells by ELISA.

Indirect immunofluorescence

By IIF, 23 of the 25 bullous pemphigoid sera bound to viable SCaBER cells in a punctate pattern (Fig. 5a). Of the remaining two sera, one showed an even, diffuse fluorescence over the

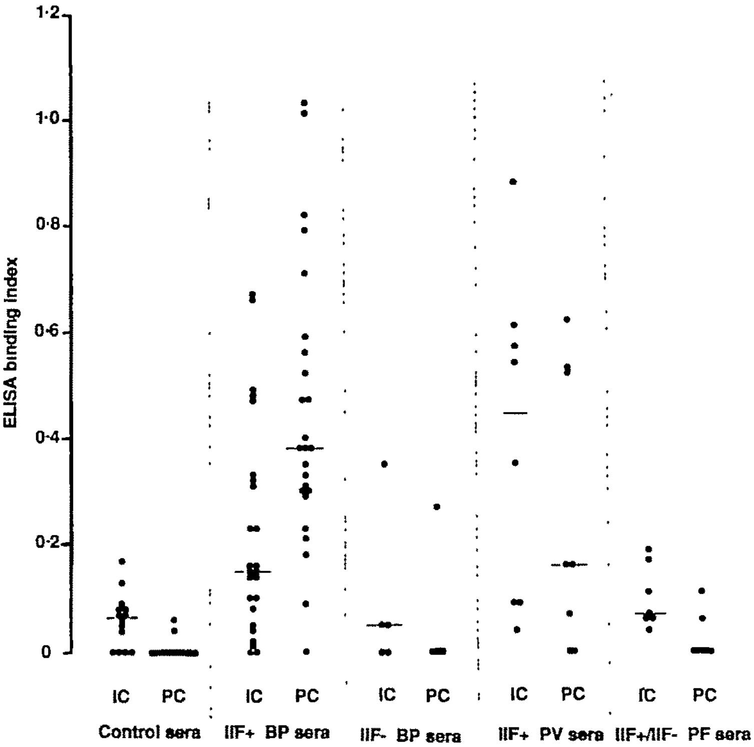

FIGURE 3. Binding of bullous pemphigoid (BP), pemphigus vulgaris (PV), pemphigus foliaceus (PF) or control sera to intact, viable SCaBER cells (IC) or cells permeabilized with 0·1% saponin (PC), by ELISA. Bars indicate medians. IIF-positive bullous pemphigoid sera showed significantly increased binding to permeabilized cells compared with that to intact cells ($P < 0·001$). All other groups of sera, including control sera, showed reduced binding to permeabilized cells compared with that to intact, viable cells.

cells and the other showed no detectable binding by IIF. The binding patterns were similar on viable and permeabilized cells (Fig. 5a and b), although some bullous pemphigoid sera showed increased binding to permeabilized cells (Fig. 5b). The IIF pattern for five of the eight pemphigus vulgaris sera appeared stronger at the edges of the cells, with a speckled appearance over the remainder of the cells, on viable cells (Fig. 5c) and permeabilized cells (Fig. 5d). The remaining sera showed an even speckled pattern over the cells. Pemphigus foliaceus sera bound weakly in an even pattern over the cells, similar to that seen with the control sera (Fig. 5e). Control sera showed high non-specific binding to permeabilized cells (Fig. 5f). Scores were given according to the intensity of fluorescence, so that strong, speckled staining would be given a score of 3, and weak diffuse binding would be given a score of 1. There was a significant correlation between the binding of sera to SCaBER cells detected by IIF and the binding indices measured by ELISA; the Spearman's rank coefficient for 30 pemphigoid sera was 0·841 ($P < 0·01$), and for 14 pemphigus sera was 0·829 ($P < 0·01$). However, no correlation was found between the serum titres by IIF on skin sections and the binding indices by ELISA, for either the pemphigoid or pemphigus sera. Some sera which were positive by IIF on skin sections did not bind in the ELISA, and some sera with low titres showed high binding to SCaBER cells.

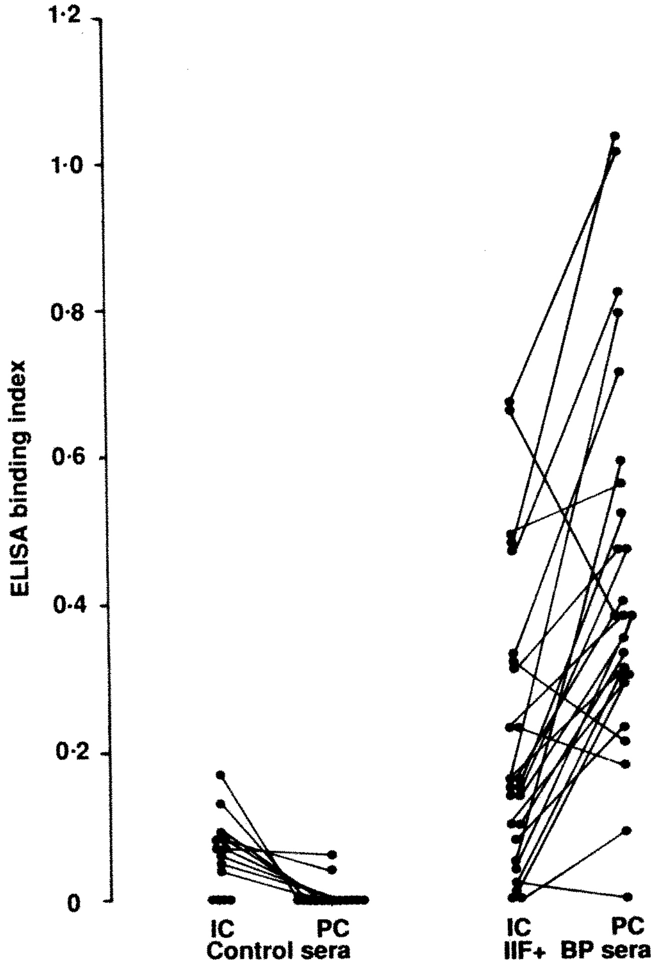

FIGURE 4. Differences in binding of individual IIF-positive bullous pemphigoid (BP) sera and control sera to intact, viable SCaBER cells (IC) or permeabilized cells (PC).

Following permeabilization with 0·1% saponin, there remained no correlation between IIF titres on skin sections and binding indices on permeabilized SCaBER cells by ELISA.

To determine if there were antigens common to SCaBER cells and normal human skin, sera from two patients with bullous pemphigoid and two patients with pemphigus vulgaris were absorbed with air-dried SCaBER cells. The IIF titres for pre-absorbed sera were reduced compared with non-absorbed sera, but total absorption of specific antibodies was not achieved with these sera.

DISCUSSION

Antibodies present in the sera of 35% of patients with bullous pemphigoid bound above control levels to SCaBER cells in an ELISA. This confirms similar findings by Bevan *et al.*,[7] who showed significant binding of 50% of bullous pemphigoid sera to this cell line by radioimmu-

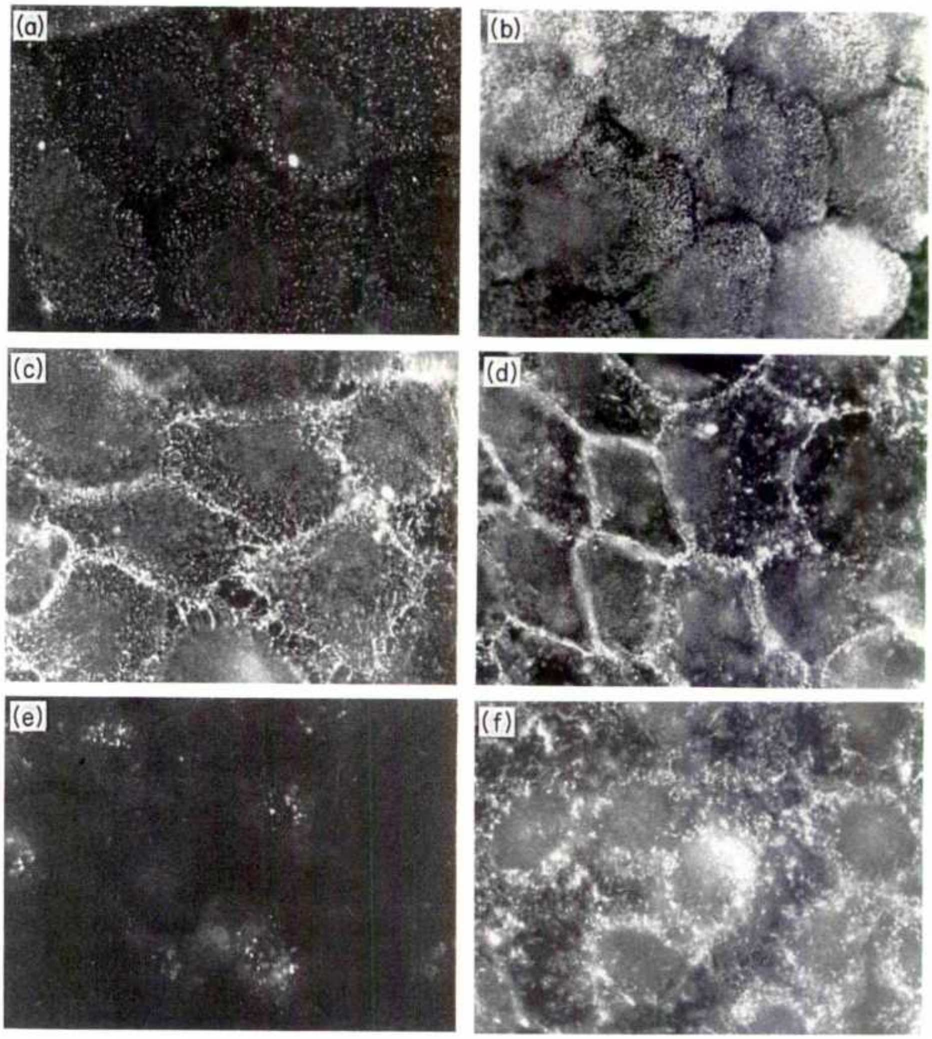

FIGURE 5. Immunofluorescence photomicrographs, showing: Speckled pattern typical of bullous pemphigoid serum binding to (a) intact, viable cells and (b) saponin-permeabilized SCaBER cells. Binding of pemphigus vulgaris serum to (c) viable cells or (d) saponin-permeabilized SCaBER cells is predominantly at the cell margins. Non-specific binding of reference control serum to (e) viable cells and (f) saponin-permeabilized SCaBER cells.

noassay. These data suggest that antigens on the SCaBER cell surface are recognized by a proportion of bullous pemphigoid sera.

No correlation was found between antibody titre detected by IIF using human skin as substrate and binding of individual sera in the ELISA using the SCaBER cell line as substrate. However, the significant correlation between binding of sera detected by ELISA and by IIF using the SCaBER cell line as substrate for both systems, suggests that the discrepancies are not due to the different detection systems, but may be due to differences in antigen expression or accessibility in the two substrates. Further studies were carried out with permeabilized cells to determine whether the differences in binding of sera were due to accessibility of antigens in air-dried skin sections or intact, viable SCaBER cells.

Previous work has shown that bullous pemphigoid antigens can be detected in mouse keratinocytes permeabilized with 0·1% saponin, distilled water or freeze-thawing, visualized by IIF.[11] Specific binding of bullous pemphigoid sera to permeabilized SCaBER cells suggests that the antigens detected by these sera are not solubilized or damaged by saponin. Bullous pemphigoid sera showed increased binding to cells permeabilized with saponin prior to the assay, suggesting that the antigens are largely intracellular. Bullous pemphigoid antigens appear to be associated with the cytoplasmic face of the hemidesmosomes in the epidermis, as demonstrated by immuno-electron microscopy.[11,12] Mutasim *et al.*[11] and Westgate *et al.*[12] also showed that bullous pemphigoid sera bound to skin sections in a punctate pattern by IIF. A similar pattern of staining was seen with the majority of bullous pemphigoid sera on SCaBER cells.

There remained no correlation between the binding of sera to permeabilized SCaBER cells by ELISA and IIF-titres on skin sections. There do appear to be some common antigens shared by these two substrates, as suggested by the partial absorption of specific antibodies. The antigens in bullous pemphigoid appear to be heterogeneous, with a range of antibodies present in patient sera. Previous work has shown variations in the levels of binding of pemphigoid sera to different substrates such as monkey oesophagus, guinea-pig oesophagus and normal human skin,[13] and different levels of binding to skin from different individuals.[14] In addition, isolated proteins recognized by bullous pemphigoid sera have a range of sizes from relative molecular mass (M_r) 9000 when extracted from normal human epidermis and purified by ion-exchange chromatography,[15] to M_r 220,000 when immunoprecipitated from normal cultured keratinocytes.[16] Antigens purified from human oesophagus by affinity chromatography in our laboratory were of M_r 68,000 and M_r 33,000.[17] Recent work has shown that sera from patients with bullous pemphigoid identify a range of bands of M_r 240,000, 220,000, 180,000, 97,000 and 77,000, by an immunoblotting technique.[18]

Antibodies from the sera of 62% of patients with pemphigus vulgaris bound above control levels in the ELISA. Binding of the majority of pemphigus vulgaris sera to viable SCaBER cells has also been demonstrated by radioimmunoassay.[7] Binding of seven pemphigus vulgaris sera to this cell line was also demonstrated by IIF.[6] Pemphigus vulgaris antigens appear to be localized extracellularly on SCaBER cells, as the binding of sera is not improved by permeabilization of the cells. However, none of the pemphigus foliaceus sera bound to SCaBER cells in the ELISA, in contrast with the radioimmunoassay of Bevan *et al.*[7] in which all four pemphigus foliaceus sera tested showed high binding.

Pemphigus vulgaris and pemphigus foliaceus sera showed no correlation between antibody titre by IIF on skin sections and binding in the ELISA to either intact or permeabilized cells. The discrepancies may be due to the different levels of antigen expression in the two substrates. Partial absorption of specific antibodies against SCaBER cells has been shown in the present study and by other authors.[6] Pemphigus sera show variations in the level of binding to different substrates such as guinea-pig lip, rabbit and monkey oesophagus, monkey lip and human skin.[13,19] In addition, antigens recognized by sera from patients with pemphigus vulgaris were of M_r 66,000 when isolated from human epidermis[20] to M_r 130,000 when immunoprecipitated from non-ionic detergent extracts of cultured keratinocytes.[21] Pemphigus foliaceus sera recognize a distinct antigen of M_r 160,000 and do not immunoprecipitate the M_r 130,000 pemphigus vulgaris antigen.[22]

The ELISA is a useful research tool for the study and quantification of antibodies in sera from patients with pemphigoid or pemphigus. To date, research and clinical interest has been focused on the measurement of the autoantibody in these diseases. However, pemphigus antigens are present in the saliva of normal individuals,[23] and both pemphigus[24] and bullous pemphigoid antigens have been detected in the urine of patients and normal individuals.[25] It is likely that these antigens are released during normal tissue turnover and the levels may be increased in a disease in which there is extensive tissue damage. Therefore, measurement of 'free' antigen or antigen-antibody complexes may be a better indicator of disease activity. Antigen is present in patients' urine and inhibition of the ELISA would be a rapid and convenient method of determining antigen levels. The assay may be of value for detection of these antigens during purification procedures in the laboratory.

The antigens detected by patients' sera in SCaBER cells, normal cultured human keratinocytes and human epidermis are being compared using an immunoblotting technique.[18] These data may help to explain the differences shown in the binding of sera to these substrates.

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REFERENCES

1 Ahmed AR, Maize JC, Provost TT. Bullous pemphigoid. Clinical and immunologic follow-up after successful therapy *Arch Dermatol* 1977; **113:** 1043–6.

2 Sams WM, Jordan RE. Correlation of pemphigoid and pemphigus antibody titre with activity of disease. *Br J Dermatol* 1971; **84:** 7–13.

3 Ahmed AR, Hombal SM. Cicatricial pemphigoid. *Int J Dermatol* 1986; **25:** 90–6.

4 O'Laughlin S, Goldman GC, Provost TT. Fate of pemphigus antibody following successful therapy. Preliminary evaluation of pemphigus antibody determinations to regulate therapy. *Arch Dermatol* 1978; **114:** 1769–72.

5 Judd KP, Lever, WF. Correlation of antibodies in skin and serum with disease severity in pemphigus. *Arch Dermatol* 1979; **115:** 428–32.

6 Ahmed AR, Murahata R. Detection of pemphigus-like antigens on tumor cell lines. *Arch Dermatol Res* 1981; **271:** 441–5.

7 Bevan HJ, Murahata RI, Ahmed AR. Detection of anti-intercellular cement substance and anti-basement membrane zone antibodies by radioimmunoassay using SCaBER tumour cell line as substrate. *Br J Dermatol* 1983; **109:** 261–5.

8 O'Toole CM, Nayak SR, Price Z *et al.* A cell line (SCaBER) derived from squamous cell carcinoma of the human urinary bladder. *Int J Cancer* 1976; **17:** 707–14.

9 Bangham AD, Horne RW. Action of saponin on biological cell membranes. *Nature* 1962; **196:** 952–3.

10 O'Toole C, Price ZH, Ohnuki Y, Unsgaard B. Ultrastructure, karyology and immunology of a cell line originated from a human transitional-cell carcinoma. *Br J Cancer* 1978; **38:** 64–76.

11 Mutasim DF, Takahashi Y, Labib RS *et al.* A pool of bullous pemphigoid antigen(s) is intracellular and associated with the basal cell cytoskeleton-hemidesmosome complex. *J Invest Dermatol* 1985; **84:** 47–53.

12 Westgate GE, Weaver AC, Couchman JR. Bullous pemphigoid antigen localisation suggests an intracellular association with hemidesmosomes. *J Invest Dermatol* 1985; **84:** 218–24.

13 Bystryn JC, Sabolinski M. Effect of substrate on indirect immunofluorescence tests for intercellular and basement membrane zone antibodies. *J Am Acad Dermatol* 1986; **15**: 973–7.

14 Zhu XJ, Bystryn JC. Heterogeneity of pemphigoid antigens. *J Invest Dermatol* 1983; **80**: 16–20.

15 Diaz LA, Calvanico NJ, Tomasi TB, Jordan RE. Bullous pemphigoid antigen: Isolation from normal human skin. *J Immunol* 1977; **118**: 455–60.

16 Stanley JR, Hawley-Nelson P, Yuspa SH, *et al.* Characterization of bullous pemphigoid antigen—a unique basement membrane protein of stratified squamous epithelia. *Cell* 1981; **24**: 897–903.

17 Pye RJ, Harkiss GD, Cawston TE. Isolation of pemphigoid antigen from normal human epidermis. *J Invest Dermatol* 1983; **80**: 364.

18 Labib RS, Anhalt GJ, Patel HP, *et al.* Molecular heterogeneity of the bullous pemphigoid antigens as detected by immunoblotting. *J Immunol* 1986: **136**: 1231–5.

19 Judd KP, Mescon H. Comparison of different epithelial substrates useful for indirect immunofluorescence testing of sera from patients with active pemphigus. *J Invest Dermatol* 1979; **72**, 314–6.

20 Peterson LL, Wuepper KD. Isolation and purification of a pemphigus vulgaris antigen from human epidermis. *J Clin Invest* 1984; **73**: 1113–20.

21 Stanley, JR, Yaar M, Hawley-Nelson P, Katz SI. Pemphigus antibodies identify a cell surface glycoprotein synthesised by human and mouse keratinocytes. *J Clin Invest* 1982; **70**: 281–8.

22 Stanley JR, Koulu L, Thivolet C. Distinction between epidermal antigens binding pemphigus vulgaris and pemphigus foliaceus autoantibodies. *J Clin Invest* 1984; **74**: 313–20.

23 Diaz LA, Patel H, Calvanico NJ. Isolation of pemphigus antigen from human saliva. *J Immunol* 1980; **124**: 760–6.

24 Murahata RI, Ahmed AR. Partial purification and characterization of pemphigus-like antigens in urine. *Arch Dermatol Res* 1983; **275**: 118–23.

25 Diaz LA, Patel H, Calvanico, NJ. Bullous pemphigoid antigen. II. Isolation from the urine of a patient. *J Immunol* 1979; **122**: 605–8.

British Journal of Dermatology (1989) **120**, 71–75.

Intestinal permeability in patients with atopic eczema

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SUMMARY

Passive intestinal permeability was measured in 15 adult patients with atopic eczema. Values in the patients did not differ significantly from those in 12 healthy control subjects. These results suggest that altered intestinal permeability is not important in the pathogensis of atopic eczema in adults.

Atopic eczema (AE), so often encountered in clinical practice, still poses considerable problems regarding its pathogenesis. Various factors can play a role in AE. A genetic predispostition is very important; the term 'constitutional eczema' is long established and widely used. While immunological factors contribute to the pathogenesis of AE, the aetiology of the disease is multifactorial and not solely allergic.[1–3]

Among the many non-immmunological factors which might produce immunological changes and thus, in turn, contribute to the pathogenesis of AE, it has been suggested that pathophysiological changes in passive intestinal permeability may occur in patients with AE. The consequent increase in the passage of macromolecules into the subepithelial layer could trigger different mechanisms contributing to the production of AE. These could include immunological mechanisms, a lectin-like action, a direct action on mastocytes and a true inflammatory action.[1–3]

Considering the possible contribution of these mechanisms to the pathogenesis of AE, it is significant that, especially in children, certain foods (for example milk, eggs and crustaceans) may cause or aggravate the symptoms of eczema, while the condition can be greatly improved by an elimination diet.[4]

The passive permeability of the small intestine can be now measured by evaluating the simultaneous intestinal absorption of the two substances, mannitol and lactulose.[5,6] While both are characterized by a low solubility in lipids, they differ in molecular weight; mannitol is a polyhydric alcohol of low molecular weight (180) while lactulose is a disaccharide with a higher molecular weight (342).

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The absorption rate of mannitol is a measure of the surface area of the small intestine, while that of lactulose is related to the surface area and permeability of the intestine. The lactulose : manitol excretion ratio (LMER) is a good indicator of abnormalities in intestinal wall permeability.

The aim of the present study was to measure the LMER in patients with AE of different degrees of severity and to correlate this value with serum IgE levels, results of RAST tests for food allergy and the extent of skin involvement.

METHODS

Fifteen patients with AE (five males, nine females) aged from 14 to 58 years (mean 32 years) were studied. All had had AE since childhood, and most since their first years of life. The skin lesions

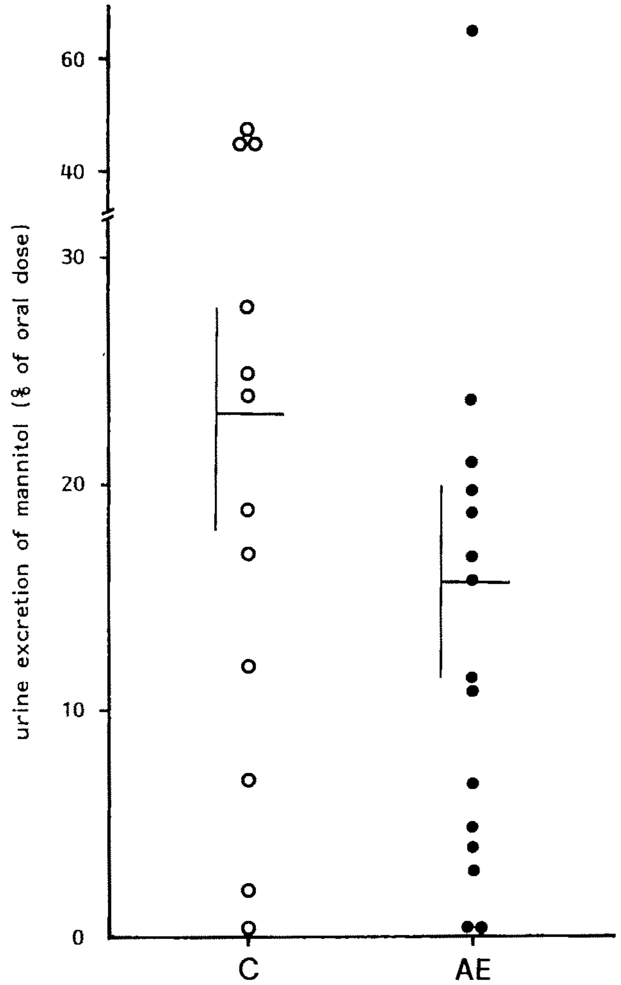

FIGURE 1. Urinary mannitol excretion in contols (C) and in patients with atopic eczema (AE). Bars represent means ± SEM.

varied in extent (measured as percentage of body surface involved) and severity (as indicated by erythema, exudation, lichenification), but in only one patient was the extent of the lesions more than 50%. Pruritus of different degrees of intensity was experienced by all patients. None of the patients had signs or symptoms of small bowel disease (nausea, vomiting, diarrhoea or steatorrhoea, recurrent abdominal pain or borborygmi). All the patients were instructed to discontinue any drugs at least 7 days before the test. In particular, anti-inflammatory agents, antihistamines and laxatives were forbidden.

Twelve control subjects (six males, six females) of similar ages to the AE patients (mean 36 years, range 16–54 years) were also studied. All the controls were free from intestinal disease, AE and other skin disorders.

All subjects gave their informed consent to the study.

After an overnight fast each subject drank 100 ml of an aqueous solution containing 0·5 g of mannitol (2·777 mmol) and 10 g of lactulose (29·239 mmol). The subjects then continued to fast for a further 3 h.

All urine passed during a 6 h period was collected and the volume measured and a sample was stored at 4°C. Lactulose and mannitol levels were measured by gas chromatography using a cross-linked methilsilicone capillary column (ultra I), i.d. 0·2 mm and film 0·33 mm, length 5 m. The temperature was programmed to rise from 100 to 230°C at 5°C/min, split 1 : 100, head pressure 10 psi.

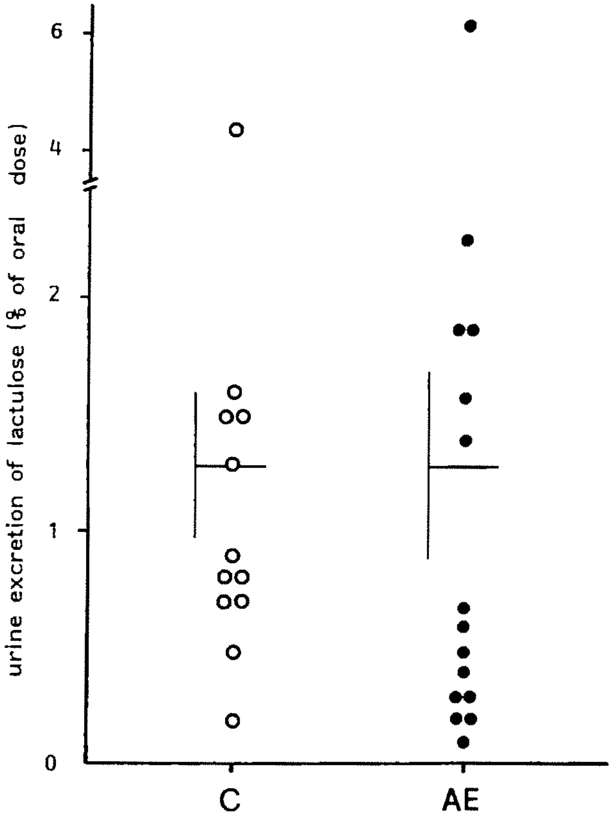

FIGURE 2. Urinary lactulose excretion in contols (C) and in patients with atopic eczema (AE). Bars represent means ± SEM.

STATISTICAL ANALYSIS

The lactulose and mannitol excretion rates and the lactulose : mannitol excretion ratios in the two groups of subjects were compared using Student's *t*-test.

To look for a possible relationship between LMER and IgE levels, RAST and extent of the skin involvement Student's *t*-test was used to compare LMER values in patients with positive and negative RAST tests, regression analysis to correlate LMER values and serum IgE levels and analysis of variance to analyse the relationship between LMER values and extent of skin involvement.

RESULTS

The individual results for mannitol excretion, lactulose excretion and LMER in controls and AE patients are shown in Figures 1, 2 and 3. No statistically significant difference was found between the two groups for any of these three parameters. No significant correlation was found between LMER values and serum IgE levels ($r = 0{\cdot}295$).

No statistically significant difference in LMER values was found between RAST positive and RAST negative patients. Analysis of the variance showed no statistically significant relationship between the extent of skin involvement and LMER values.

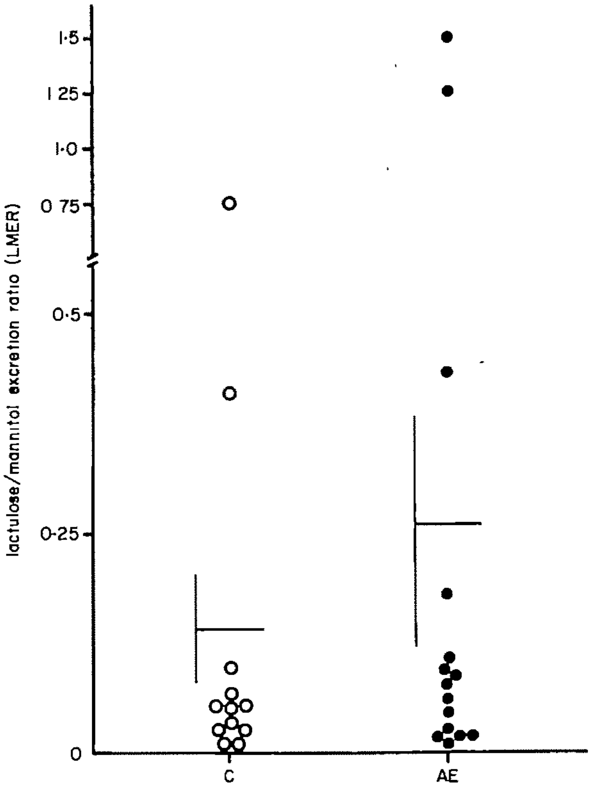

FIGURE 3. Lactulose/mannitol excretion ratios in contols (C) and in patients with atopic eczema (AE). Bars represent means ± SEM.

DISCUSSION

In the present study of passive intestinal permeability, measured by mannitol and lactulose excretion, only a slight difference was found between eczematous and healthy subject, and this difference was not statistically significant. Our results differed from those of Ukabam *et al.*[6] who reported that passive permeability of the small intestine was increased in more than 40% of their patients (5 of 11). The study by Ukabam *et al.*[6] was comparable to ours, with respect to patient selection criteria (adult patients with lesions at the time of the study), test substances and laboratory methods, but differed in the extent of the lesions (five patients with more than 50% body surface involvement), the smaller number of patients studied, and some small details of laboratory technique.

Our findings lead us to the same conclusion as was reached in two more recent studies,[7,8] namely that there is no significant abnormality in passive permeability of the small intestine in adult AE patients.

On the other hand, according to Pike *et al.*[9] changes in intestinal permeability can be more easily detected in childhood, since at an early age, even in normal subjects, an increase in intestinal permeabiltiy is a common occurrence. With increasing age, the target organ in AE patients appears to change from the digestive tract, which gradually acquires great defensive capabilities, to the respiratory system, the function of which is more severely affected in adolescents and adults.

Therefore, in order to obtain a more reliable evaluation of the relationship between intestinal permeability and atopic eczema studies need to be carried out in young children.

The results of the present study suggest that alterations in passive permeability of the small intestine do not play a key role in the pathogenesis of atopic eczema, at least in adult patients.

REFERENCES

1 Brostoff J, Challacombe SJ. Clinics in Immunology and Allergy. In: *Food Allergy* vol. 2, N° 1. Philadelphia: W.B.Saunders Co, 1982. 15–32.

2 Furokawa CT. Recent immunologic findings relating food allergy to atopic dermatitis. *Ann Allergy* 1979; **42:** 207–10.

3 Nespoli L, Guarnaccia S. Patogenesi dell'atopia. *Bol dermatol Pedia* 1983; 2 (Suppl. 3): 61–6.

4 Jackson PG, Lessoff MH, Baker RWR *et al.* Intestinal permeability in patients with eczema and food allergy. *Lancet* 1981; **i:** 1285.

5 Ukabam SO, Clamp JR, Cooper BT. Abnormal small intestinal permeability to sugars in patients with Crohn's disease of the terminal ileum and colon. *Digestion* 1983; **27:** 70.

6 Ukabam SO, Mann RJ, Cooper BT. Small intestinal permeability to sugars in patients with atopic eczema. *Br J Dermatol* 1983; **110:** 649–52.

7 Bjarnason I, Goolamali SK, Levi AJ, Peters TJ. Intestinal permeability in patients with atopic eczema. *Br J Dermatol* 1985; **112:** 291–7.

8 Bjarnason I, Peters TI, Levi AJ. Intestinal permeability: clinical correlates. *Dig Dis* 1986; **4:** 83–92.

9 Pike MG, Heddle RJ, Boulton P *et al.* Increased intestinal permeability in atopic eczema. *J Invest Dermatol* 1986; **87:** 101–4.

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An epidemiological comparison between occupational and non-occupational hand eczema

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SUMMARY

Thirty-four per cent of 2110 patients with eczema attending a contact dermatitis clinic had hand eczema. Fifty-five per cent (395 of 721) had contact dermatitis and 45% (326 of 721) had endogenous/unclassifiable eczema. Thirty-five per cent of cases (217 of 721) were occupational eczema. An epidemiological comparison of patients with occupational and non-occupational hand eczema (defined as eczema occurring on the hands up to the wrist line) was made. Hand eczema was more common in males in both groups, and there was a significantly larger proportion of males in the occupational group (65%) than the non-occupational group (51%) ($P < 0{\cdot}001$). The prevalence of a personal or family history of atopy was significantly lower in the occupational group (7%) than the non-occupational group (15%) ($P < 0{\cdot}005$). Irritant contact dermatitis occurred in a larger proportion of patients in the occupational group (76%) than the non-occupational group (39%) ($P < 0{\cdot}0001$). There was no significant difference between the proportions of patients with positive patch test reactions in the occupational group (34%) and the non-occupational group (41%). Potassium dichromate, and epoxy resin allergy occurred significantly, more frequently, in the occupational group whereas fragrance-mix allergy was significantly more frequent in the non-occupational group.

Hand eczema is a common dermatological condition, and treating it is a challenge because it is not a single entity, but a condition with many possible causes. It includes contact dermatitis and endogenous eczema. Physical examination alone is not helpful in elucidating the cause of hand eczema, as no distribution of eczema was typically allergic, irritant or endogenous.[1] A detailed history and patch testing is necessary in arriving at a diagnosis. Endogenous hand eczema is often diagnosed after the exclusion of contact dermatitis. Hand eczema is often occupational or is aggravated by working conditions, and the aim of the present study was to identify epidemiological differences between occupational and non-occupational hand eczema.

METHODS

All patients attending the contact dermatitis clinic of Middle Road Hospital, Singapore, with

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eczema, between January 1985 and December 1986 were included in the study. Patients with hand eczema (defined as eczema confined to the hands, up to the wrist line) were patch-tested with the International Contact Dermatitis Research Group (ICDRG) standard series and additional allergens where indicated, using Finn Chambers and Scanpor tape. Reading and recording of the patch-test reaction were performed according to the method described by Cronin.[2] The age, sex, history of atopy, patch-test results, diagnosis and relevant occupational details were recorded. Patients with eczema on other parts of the body in addition to the hands were excluded from the analysis.

The diagnosis of allergic contact dermatitis was based on history and a relevant positive patch test and that of irritant contact dermatitis on the history and negative patch-tests. 'Endogenous/unclassifiable eczema' referred to all eczema other than allergic and irritant contact dermatitis. It inevitably included some contact dermatitis in which the relevant irritant and allergic factors were missed. When allergic and irritant contact dermatitis co-existed, the condition was classified as allergic contact dermatitis. Occupational dermatitis was defined as eczema caused by or aggravated by the work environment.[3] Atopic conditions included atopic dermatitis, asthma and hay fever.

The Chi-square test, Fisher's exact test and Student's t-test were used for statisical analysis. This is the second part of a study on hand eczema. The first part has been reported earlier.[4]

RESULTS

Two thousand one hundred and ten patients were included in the study. Thirty-four per cent (721 of 2110) had hand eczema (mean age 32 years, range 8–81 years). Thirty-one per cent of cases (217 of 721) were occupational eczema (mean age 30 years, range 16–67 years); 69% (504/721) were non-occupational eczema (mean age 33 years, range 8–81 years). The patients with occupational eczema were significantly younger than those with non-occupational eczema ($P < 0·001$).

The numbers of patients with occupational and non-occupational hand eczema are shown in Table 1 in relation to age group and sex. There were more males than females in both the occupational and non-occupational groups. There were significantly more males in the occupational group (65%) than in the non-occupational group (51%) ($P < 0·005$).

TABLE 1. Numbers of patients with occupational and non-occupational hand eczema in relation to age group and sex

	Occupational hand eczema			Non-occupational hand eczema		
Age group (years)	Males no. (%)	Females no. (%)	Total no. (%)	Males no. (%)	Females no. (%)	Total no. (%)
< 20	11 (7·7)	11 (14·7)	22 (10·1)	25 (9·5)	27 (11·1)	52 (10·3)
20–29	72 (50·7)	40 (53·3)	112 (51·6)	99 (37·8)	110 (45·5)	209 (41·5)
30–39	34 (23·9)	17 (22·7)	51 (23·5)	61 (23·3)	61 (25·2)	122 (24·2)
40–49	8 (5·6)	4 (5·3)	12 (5·5)	30 (11·5)	20 (8·3)	50 (9·9)
50–59	11 (7·7)	3 (4·0)	14 (6·5)	24 (9·2)	11 (4·5)	35 (6·9)
> 59	6 (4·2)	0 (0)	6 (2·8)	23 (9·8)	13 (56·4)	36 (7·1)
Total	142 (100%)	75 (100%)	217 (100%)	262 (100%)	242 (100%)	504 (100%)

The numbers of patients with different types of hand eczema are shown in Table 2. Thirty-two per cent of patients (233 of 721) had irritant contact dermatitis, 23% (162 of 721) had allergic contact dermatitis and 45% (326 of 721) endogenous/unclassifiable eczema.

In the non-occupational group, 64% of patients (326 of 504) had endogenous/unclassifiable eczema and 36% (178 of 504) had contact dermatitis. Irritant contact dermatitis occurred in a significantly greater proportion of patients in the occupational group (76%) than in the non-occupational group (39%) ($P < 0{\cdot}0001$). In the two groups there was no significant difference in the proportions of patients with irritant contact dermatitis between males and females.

A personal or family history of atopy was significantly less frequent in the occupational group (7%; 15 of 217) than in the non-occupational group (15%; 78 of 504), ($P < 0{\cdot}005$) in each group there was no significant difference in the proportion of patients with each diagnosis who had a personal or family history of atopy.

The prevalence of contact allergy to some allergens of the standard ICDRG series in patients with occupational and non-occupational hand eczema is shown in Table 3. Ninety-two (34%) patients in the occupational and 206 (41%) in the non-occupational group were sensitive to one or more patch. The difference between the two groups was not significant. The prevalences of potassium dichromate and epoxy resin allergy were significantly higher in patients with occupational hand eczema ($P = 0{\cdot}001$ for both; Fisher's exact test). The prevalence of fragrance-mix allergy was significantly higher in patients with non-occupational hand eczema ($P = 0{\cdot}001$).

Fifty-five per cent (53 of 92) of patients in the occupational and 53% (109 of 206) in the non-occupational group had relevant positive patch test reactions. The difference between the two

TABLE 2. Diagnoses in patients with occupational and non-occupational hand eczema

	No. of patients					
	Occupational			Non-occupational		
	Male	Female	Total	Male	Female	Total
Allergic contact dermatitis	37 (26%)	16 (21%)	53 (24%)	43 (57%)	66 (65%)	109 (61%)
(atopy)			3 (6%)			17 (15%)
Irritant contact dermatitis	105 (74%)	59 (79%)	164 (76%)	33 (43%)	36 (35%)	69 (39%)
(atopy)			12 (7%)			8 (12%)
Total contact dermatitis	142 (100%)	75 (100%)	217 (100%)	76 (100%)	102 (100%)	178 (100%)
Endogenous/ unclassifiable eczema	0	0	0	186	140	326
(atopy)						53 (13%)
Total	142	75	217	262	242	504
(atopy)			15 (7%)			78 (15%)

TABLE 3. The prevalence of contact allergy to some common allergens in the standard ICDRG series in patients with occupational and non-occupational hand eczema

	Occupational hand eczema			Non-occupational hand eczema			
Allergen	Males ($n = 142$)	Females ($n = 75$)	Total ($n = 217$)	Males ($n = 262$)	Females ($n = 242$)	Total ($n = 504$)	P
Nickel sulphate 5%	7	16	23 (8%)	18	49	67 (13%)	NS
Potassium dichromate 0·5%	15	0	15 (6%)	7	1	8 (2%)	0·001
Cobalt chloride 1%	7	4	11 (4%)	10	9	19 (4%)	NS
Epoxy resin 1%	3	4	7 (3%)	0	0	0 (0%)	0·001
PTBP resin 1%	1	0	1 (·5%)	0	0	0 (0%)	NS
Colophony 20%	7	0	7 (3%)	16	12	28 (6%)	NS
Fragrance mix 8%	9	9	18 (7%)	46	46	92 (18%)	0·001
Balsam of Peru	3	1	4 (1%)	11	15	26 (5%)	NS
p-phenylenediamine 1%	4	4	8 (3%)	15	15	30 (6%)	NS
Neomycin 20%	6	1	7 (3%)	15	18	33 (7%)	NS
Proflavine 0·5%	11	2	13 (5%)	15	8	23 (5%)	NS
Clioquinol mix 6%	2	1	3 (1%)	10	2	12 (2%)	NS
Wool alcohol 30%	4	1	5 (2%)	4	5	9 (2%)	NS
Thiuram mix 1%	2	0	2 (1%)	1	5	6 (1%)	NS
Formalin 2% aqueous	2	0	2 (1%)	1	4	9 (2%)	NS

NS = not significant.

groups was not significant. In the two groups the prevalence of relevant positive patch-test reactions did not differ significantly between males and females.

DISCUSSION

The skin of the hands is intensively exposed to irritants and allergens, and contact dermatitis is common. An occupational agent is solely or partly responsible in some cases of hand eczema while in some no definite exogenous factor appears to be responsible. These cases are often classified as endogenous/unclassifiable hand eczema. Hand eczema is common and is frequently a disabling condition. It has been found to account for about 30% of patients with eczema in Europe,[5] 21% in the U.K.[6] and 34% in Singapore.[4] There appear to be some epidemiological differences between occupational and non-occupational hand eczema.

Thirty per cent of our patients with hand eczema had occupational eczema. This proportion was higher than that reported by Agrup[7] in Sweden, where only 11% of cases of hand dermatoses were occupational eczema. Occupational eczema is a preventable disease. It has important socio-economic implications, being an important cause of absence from work.[8–10] Early diagnosis of occupational eczema can prevent morbidity and absence from work. The mean age of patients with occupational hand eczema was significantly lower than that of patients with non-occupational hand eczema ($P < 0·001$). Their age range was also narrower. The mean age of industrial workers is probably lower than that of the general population in Singapore and the age range is probably narrower, and this probably accounted for the lower mean age and narrower age range in the occupational hand eczema group. More than 50% of our patients with occupational hand eczema were between 20 and 29 years old. This is comparable to the previously reported peak age group of patients with occupational eczema in Singapore.[11] All young patients with hand eczema should be investigated for occupational eczema.

In our hand eczema patients there were more males (56%) than females (44%). Earlier reports from Sweden and Denmark showed a female preponderance.[12,13] The high prevalence in females has generally been attributed to the high incidence of 'housewives' dermatitis. Housewives are exposed to numerous irritants in domestic work and are vulnerable to contact hand dermatitis.[6] Cronin[1] also noted a higher prevalence of non-endogenous hand eczema in housewives than in working women. One possible reason for our finding of a smaller proportion of women than men among our hand eczema patients may be the high proportion of working females in Singapore. Singapore has a large female workforce in her electronics and electrical industry. Most are employed in dry jobs. Another reason could be the mechanization of numerous domestic tasks. Washing machines, dishwashers, vacuum cleaners and floor cleaners and polishers are readily available and reduce the need for housewives to expose their hands to irritants.

Fifty-five per cent of our patients with hand eczema had contact dermatitis. Among these, irritant contact dermatitis was more common (59%) than allergic contact dermatitis (41%). Similar proportions have been reported in the U.K.[1] and Sweden.[7] These findings confirm the necessity of looking for exogenous factors in patients with hand eczema. The high proportion of patients with allergic contact dermatitis (especially in the non-occupational group) also indicates the importance of patch-tests in hand eczema. Irritant contact dermatitis was significantly more frequent in the occupational hand eczema group (76%) than in the non-occupational hand eczema group (39%). It appears that most workers with occupational hand eczema, can, after rehabilitation, return to their original job. A job change often causes socio-economic hardship, and should never be recommended unless absolutely necessary.

Forty-five per cent of all cases of hand eczema (65% of those with non-occupational hand eczema) were endogenous. Some of these patients could have had contact dermatitis where the agent responsible had not been identified. Atopics appear to be more prone to occupational irritant contact dermatitis.[7,12,13] They also tend to suffer more severe occupational hand dermatitis.[13] Other constitutional factors are also known to play an important role in irritant contact dermatitis, and the presence of such factors may not necessarily be indicated by the presence of atopic disorders.[14] The overall prevalence of a personal or family history of atopy in our patients (13%) was lower than those reported in the U.K. and Sweden. Unreliable histories from our patients could be a possible explanation for this.[4] The prevalence of atopy was significantly lower in the occupational group (7%) than the non-occupational group (14%). Patients with occupational hand eczema might represent a selected group. There were probably fewer atopics among patients with occupational hand eczema, because atopics tend to suffer severe contact dermatitis and would generally tend to select dry jobs and, therefore, be less likely to develop contact dermatitis. The prevalence of atopy in the non-occupational hand eczema group (15%) was similar to that in patients with non-hand eczema (13%).[4]

About half of our patients in both groups had unexplained positive patch-test reactions. One reason could be our failure to recognize hidden sources of allergens. Another could be oral ingestion of allergens.[15–18] However, the role of systemic ingestion of contact allergens in the causation of hand eczema is still controversial.

Fragrance-mix, nickel sulphate, medicaments, chromate and epoxy resin are common contact allergens. Chromates and epoxy resin were found to be predominantly occupational allergens; they are generally encountered in the construction and electronic industries, respectively.

Fragrance-mix appeared to be predominantly an allergen in patients with non-occupational hand dermatitis. Fragrances are ubiquitous, being present in cosmetics and most domestic products. The prevalence of allergy to other common environmental allergens including nickel and medicaments was slightly higher in the non-occupational hand eczema group. These allergens should be sought in all patients with hand eczema.

REFERENCES

1 Cronin E. Clinical patterns of hand eczema in women. *Contact Dermat* 1985; **13:** 153–61.

2 Cronin E. *Contact Dermatitis.* Edinburgh: Churchill Livingstone, 1980; 8.

3 Lane G *et al.* Industrial dermatoses: report by committee on industrial dermatoses, of Section on Dermatology and Syphilogy of the American Medical Association. *J Am Med Assoc* 1942; **118:** 613–5.

4 Goh CL. An epidemiological comparison between hand eczema and non-hand eczema. *Br J Dermatol* 1988; **118:** 797.

5 Fregert S, Jhorth N, Magnusson B *et al.* Epidemiology of contact dermatitis. *Trans St John's Hosp Dermatol Soc (Lond)* 1969, **55:** 17–21.

6 Burton JL, Rook A, Wilkinson. In: *Textbook of Dermatology*, 4th Edition (Rook A, Wilkinson DS, Ebling FJG *et al.*, eds). Oxford: Blackwell Scientific Publications, 1986; 403.

7 Agrup G. Hand eczemas and other dermatoses in South Sweden. *Acta Dermatovenereol* 1969; **49** (Suppl 61) 28–38.

8 Taylor PJ. Sickness absence. Facts and misconceptions. *J R Coll Phys (Lond)* 1974; **8:** 315–33.

9 Newhouse ML. Sickness absence due to industrial dermatitis. *Trans St John's Hosp Dermatol Soc (Lond)* 1972; **58:** 48–50.

10 Goh CL. Sickness absence due to occupational dermatoses in a prefabrication construction factory. *Contract Dermat* 1986, **15:** 28–31.

11 Goh CL. Occupational skin disease in Singapore: epidemiology and causative agents. *Ann Acad Med Singapore* 1987; **16:** 303–5.

12 Rystedt I. Atopic background in patients with occupational hand eczema. *Contact Dermat* 1985; **12:** 247–54.

13 Nilsson E, Mikaelsson B, Andersson S. Atopy, occupation and domestic work as risk factors for hand eczema in hospital workers. *Contact Dermat* 1985; **13:** 216–23.

14 Hjorth N, Avnstorp C. Rehabilitation in hand eczema. *Dermatosen* 1986; **34:** 74–6.

15 Fisher AA. Possible role of diet in pompholyx and nickel dermatitis—a critical survey. *Cutis* 1978; **22:** 412–4.

16 Christensen OB, Moller H. External and internal exposure to the antigen in the hand eczema of nickel allergy. *Contact Dermat* 1975; **1:** 136–41.

17 Kaaber K, Veien NK. Chromate ingestion in chronic chromate dermatitis. *Contact Dermat* 1978; **4:** 119–20.

18 Veien NK, Hattel T, Justensen O, Norholm N. Oral challenge with balsam of Peru. *Contact Dermat* 1985; **12:** 104–7.

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Dapsone as first line therapy for bullous pemphigoid

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SUMMARY

In an open study, a total of 18 patients with proven bullous pemphigoid (16 new patients and two in relapse) were treated with a trial of dapsone (17 patients) or sulphonimide (one patient). Overall, eight patients were controlled on one of these agents as the sole treatment (44%). Six patients had a partial, but inadequate response, while four did not respond. The responses to the sulpha drugs were generally rapid, i.e. within 2 weeks, and the maximum doses used in any patient were 100 mg/day dapsone and 1·5 g/day of sulphapyridine or sulphamethoxypyridazine. Significant side-effects to dapsone occurred in six of the 17 patients. A trial of a sulphone or sulphonamide drug is warranted in bullous pemphigoid both as an initial treatment or in the treatment of relapse, particularly when there is a contraindication to the use of corticosteroids. Side-effects are common and therapy needs to be closely monitored.

Bullous pemphigoid (BP) is a chronic blistering disease for which prednisolone is regarded as the treatment of choice. However, as the patients affected by the disease tend to be elderly, and the doses of prednisolone required are sometimes high, there may be considerable morbidity associated with the treatment. Immunosuppressive drugs, particularly azathioprine, are frequently used in addition to prednisolone, as steroid-sparing agents,[1] although, in our experience, these are not always well tolerated. Furthermore, in some patients there may be a pre-existing contraindication to the use of steroids, e.g. osteoporosis, diabetes mellitus or hypertension, or to immunosuppressives, e.g. a history of malignant disease, and in these patients alternative first line treatments would be useful.

Both dapsone and sulphapyridine have been reported to be effective in some pemphigoid patients.[2–4] Person and Rogers[2] found dapsone or sulphapyridine effective at controlling the disease in six out of 41 patients, while Piamphongsant[4] found dapsone effective in 13 out of 28 patients. In order to assess the efficacy of dapsone as a first line treatment in BP, it has been our practice in recent years to use a trial of dapsone or a sulphonamide as the initial treatment in patients presenting with bullous pemphigoid, only adding or substituting prednisolone if this

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TABLE 1. Details of 18 bullous pemphigoid patients treated with dapsone or a sulphonamide

Patient no.	Sex	Age (years)	IMF findings direct	IMF findings indirect	Neutrophil rich inflammatory infiltrate	Maximum dose and outcome (dapsone unless otherwise stated)
1	F	72	IgG,C_3	−ve	+++	100 mg/day Total rem. < 2 weeks
2	F	85	IgG,C_3	−ve	+++	100 mg/day Total rem. < 2 weeks
3	F	67	C_3	1:640	−	100 mg/day Total rem. < 2 weeks
4	F	80	IgG,C_3	−ve	−	100 mg/day Total rem. < 2 weeks
5	M	74	IgG,C_3	1:40	−	100 mg/day Total rem. < 2 weeks
6	F	89	IgG,C_3	1:80	+++	50 mg/day Total rem. 3–4 weeks
7	F	69	IgG,C_3	1:80	−	100 mg/day Total rem. 3–4 weeks
8	M	75	IgG,C_3	1:80	−	100 mg/day Partial rem. only
9	F	68	C_3	1:40	−	100 mg/day Partial rem. only
10	M	83	IgG	1·40	++	1·5 g/day sulph. Partial rem. only
11	F	71	IgG,C_3	1:40	−	100 mg/day Partial rem. only
12	F	69	IgG,C_3	1:40	−	100 mg/day No response
13	F	66	IgG,C_3	1:320	++	100 mg/day No response
14	F	49	C_3	−ve	−	100 mg/day No response SMP—1·5 g/day
15*	F	82	IgG,C_3	1:640	−	100 mg/day Pred. 2 mg/day Partial rem. only
16*	M	65	IgG	1:320	−	100 mg/day pred 10 mg/day Partial rem. only
17**	M	73	IgG,C_3	1:640	−	Pred. 40 mg/day aza. 100 mg/day
18**	M	83	IgG	1:160	−	Pred. 40 mg/day

* These two patients were already receiving prednisolone, Patient 15 for the treatment of rheumatoid arthritis and Patient 16 for polymyalgia rheumatica.

** Patients treated in relapse.

TABLE I (*cont.*)

Side-effects (dapsone unless otherwise stated)	Subsequent course	Length of follow-up
haemolytic anaemia	Succesfully treated with SMP for 3 years. Remained in rem.	3 years 10 months
none	Dapsone d/c after 3 months Remained in rem. off all treatment	1 year 4 months
none	In rem. on 100 mg/day dapsone Relapsed if dose lowered	1 year
none	Dapsone d/c after 3 months Remained in rem. on no treatment	9 months
none	In rem. on 100 mg/day dapsone Died after 1 month of myocardial infarction.	2 months
moderate haemolysis	Dapsone d/c after 1 year Remained in rem.	2 years
none	Dapsone d/c after 1 year Remained in rem.	2 years 9 months
none	Controlled on Pred. 20 mg/day and dapsone.	3 years 3 months
moderate haemolysis	Pred. 30 meg/day added with rapid rem.	6 weeks
Agranulocytosis and drug eruption	Controlled on Pred. 40 mg/day	2 years 3 months
none	Controlled on Pred. 40 mg/day and dapsone	3 years 1 month
none	Controlled on Pred. 30 mg/day	2 years
lethargy neutropaenia	Controlled on Pred. 30 mg/day	2 years
haemolytic anaemia (dapsone) nausea (SMP)	No response to SMP Controlled on Pred. 60 mg/day and aza 150 mg/day	2 years 2 months
none	Controlled on Pred. 30 mg/day alone	4 months
none	Controlled on Pred. 35 mg/day and dapsone	2 years 5 months
headaches	Generalized relapse controlled with dapsone and then SMP	11 years 11 months
none	Repeatedly relapsed when Pred. reduced to 15 mg/day. Dapsone 100 mg/day allowed slight reduction of Pred. dosage.	3 years 4 months

Pred. = prednisolone; sulph = sulphapyridine, SMP = sulphamethoxypyridazine; aza = azathioprine; rem. = remission; d/c = discontinued.

failed to control the disease. We present here a review of our experience with this practice in 15 patients who received dapsone, and one treated with sulphapyridine, and we also report the results of treatment in two further patients in whom relapses of bullous pemphigoid were managed with one of these agents.

METHODS

Patients

Eighteen patients (six men and 12 women; age range 49–89 years, mean age 73 years) who presented with acute blistering and pruritus, had a diagnosis of bullous pemphigoid confirmed by established clinical, histopathological and immunopathological criteria. Patient details are given in Table 1. The patients were studied prospectively, with a mean follow-up period of 2 years 6 months (range 6 weeks–11 years 11 months). All the patients had widespread blistering affecting the limbs and trunk, except for two whose blisters were localised to the lower legs. Six patients also had lesions of the mucous membranes. In all patients, IgG or C3 were detected by immunofluorescence (IF) in the basement membrane zone of uninvolved skin. IgA deposition was not found in the skin, but in one patient (Patient 1) deposition of IgA was found in the BMZ in a lip biopsy. Indirect IF was positive in 13 of 17 patients. The patients studied were unselected, and did not differ from the total group of 65 patients with bullous pemphigoid seen over the previous 7 years, with respect to age, extent of disease, mucous membrane involvement or indirect immunofluorescence findings.

Treatment

Dapsone was used as the initial treatment in 15 patients presenting for the first time. In 13 patients dapsone was the sole initial treatment, while in two patients, (Patients 12 and 13) it was added to a low dose of prednisolone, already being taken long-term for the management of rheumatoid arthritis and polymyalgia rheumatica, respectively. One patient was treated with sulphapyridine as the sole initial treatment in a starting dose of 0·5 g daily, increasing in 0·5 g/day increments to 1·5 g/day.

A further two patients received dapsone in the treatment of a relapse of bullous pemphigoid (Patients 17 and 18). One patient had been initially treated with prednisolone and had relapsed repeatedly when the dose was lowered to 15 mg daily. Dapsone was therefore used as an adjunctive therapy. The other patient had initially been successfully treated with prednisolone and azathioprine, and had been in remission, off all treatment, for 6 years before developing a relapse of widespread blistering, which was treated with dapsone.

Dapsone was started at a dose of 50 mg/day and increased by 50 mg/day increments after 5–7 days if the clinical response was inadequate, and provided that there was no evidence of significant haemolysis or other side-effects.

In addition to systemic treatment, all patients were also treated with topical clobetasol proprionate (Dermovate^{TM}) applied to any itchy urticated or blistering lesions.

A response to treatment was considered complete if there was complete abolition of fresh blisters or urticated lesions, and if any localized areas of irritation could be readily controlled with the use of topical treatment. A reduction in the number of fresh lesions was considered a partial response.

Histological evaluation

Histological sections taken from fresh blisters from 14 patients were examined by an independent observer, and the relative numbers of mononuclear cells, eosinophils and neutrophils in the dermal infiltrate were assessed. The intensity of the neutrophil infiltrate was graded on a scale from 0 (=no neutrophils present) to + + + + (neutrophils constituting >90% of the inflammatory infiltrate). An attempt was made to relate the nature of the inflammatory infiltrate to the response to treatment.

RESULTS

The results are summarized in Tables 1 and 2.

Patients presenting de novo

Initial response. Five of the 15 patients treated initially with dapsone showed a complete response to treatment within 2 weeks of starting treatment. A further two patients showed a more gradual, but eventually complete response over 3–4 weeks. The maximum dose of dapsone required in the responders was 100 mg daily. A partial remission was achieved in a further four patients treated with dapsone and in the one patient treated with sulphapyridine, while four of the dapsone treated patients showed no appreciable response.

Subsequent course. Of the seven complete responders, one patient died of a myocardial infarction, 1 month after starting treatment. He had no evidence of either haemolysis or methaemoglobinaemia. Four patients continued on dapsone with complete control of their disease until the drug was gradually withdrawn after a period of 3–12 months. All four remained in remission without treatment after follow-up periods of 9 months to 2 years 4 months (mean 19 months). One patient's disease remained completely controlled for 1 year on 100 mg dapsone

TABLE 2. Summary of treatments and outcome

	No. of patients			
	Total no. of patients	Initial response	Side-effects	Subsequent treatment
Dapsone as initial sole therapy	13	Complete 7	2	6 dapsone alone 1 SMP alone
		Partial 2	1	2 dapsone and prednisolone
		None 4	2	1 SMP (ineffective and not tolerated) so controlled on prednisolone and azathioprine 1 minocin (ineffective) so controlled on prednisolone alone 2 dapsone and prednisolone
Dapsone added to long-term, low-dose prednisolone as initial treatment	2	Partial 2	0	1 dapsone and prednisolone 1 prednisolone alone
Sulphapyridine as initial sole therapy	1	Partial 1	1	1 prednisolone alone
Patients in relapse treated with dapsone	2	Complete 1	1	1 SMP effective and well tolerated
		Partial 1	0	1 dapsone and prednisolone

daily, but relapsed when the dose was lowered. One patient showed a rapid and complete response to dapsone, but developed haemolytic anaemia and was changed to sulphamethoxypyridazine (1 g daily) which also controlled her disease, apart from occasional mouth lesions. Treatment was withdrawn after 3 years, and she remained in remission 10 months later.

Of the four patients who partially responded to dapsone, two were subsequently controlled by the addition of prednisolone while continuing to take dapsone. One of these developed moderate haemolysis precluding any further increase in dapsone dosage. At the time of writing this patient had been treated for a short period only and was receiving dapsone 100 mg/day prednisolone 30 mg/day. The other patient was controlled on dapsone 100 mg/day and prednisolone (maximum dose 20 mg/day), until dapsone was discontinued after 6 months of treatment and prednisolone after 2 years 9 months. He remained in remission without treatment 6 months later. The other two partial responders were already receiving long-term prednisolone, 2 mg daily for rheumatoid arthritis and 10 mg daily for polymyalgia rheumatica respectively, when they developed bullous pemphigoid. These two, therefore, were treated by adding dapsone (50 mg increasing to 100 mg daily) to their steroids. Neither showed a complete response to dapsone and their steroid doses were, therefore, increased to 30 mg and 35 mg, respectively, with rapid control of the disease. One patient discontinued the dapsone inadvertently, at the time of writing had been contolled on 10 mg prednisolone daily for 4 months. The other patient continued to require 100 mg dapsone daily in addition to 10 mg prednisolone daily to control his symptoms, after 2 years of follow-up.

The one patient who received suphapyridine as initial treatment, showed only a partial response to 1·5 g/day; the addition of prednisolone 40 mg/day brought about a prompt remission. However, he developed a fall in white blood cell count and a severe drug eruption after 3 weeks on sulphapyridine, necessitating withdrawal of the drug. In spite of discontinuing sulphapyridine the white cell count continued to drop to a total of $0{\cdot}6 \times 10^9/l$ (neutrophils $0{\cdot}1 \times 10^9/l$). The patient was seriously ill for 2 weeks, but eventually made a full recovery from the agranulocytosis.

Non-responders. Four patients showed no appreciable response to dapsone. Two were managed by the addition of prednisolone, while continuing to take dapsone (Patients 11 and 12). The other two non-responders had side effects which necessitated discontinuing the drug. One, who developed dapsone-induced haemolytic anaemia, was subsequently tried on sulphamethoxypyridazine (maximum dose 1·5/day), which caused severe nausea and was without benefit. This patient was then controlled on prednisolone and azathioprine.

Dapsone and sulphapyridine in the treatment of bullous pemphigoid relapse

Two patients were treated during a relapse of the disease. The first of these had been controlled on prednisolone for 2 years, but relapsed each time the dose was lowered below 15 mg/daily. Dapsone 100 mg daily was added to his treatment, allowing a modest reduction in his daily steroid dose to 10 mg. The other patient had been successfully treated with prednisolone and azathioprine when he initially presented in 1976. After 2 years in remission, treatment had been discontinued. He remained in remission for a further 6 years before having an acute relapse with widespread blistering. This relapse was treated with sulphamethoxypyridazine 1·5 g daily, bringing about a prompt response. Withdrawal of this treatment after 2 years was followed by a further relapse and he was then maintained in remission on sulphamethoxypyridazine for a further 2 years until his death from unrelated causes.

Relationship of histological findings to clinical response

Most of the patients had a mixed inflammatory infiltrate, composed of eosinophil polymorphs, mixed with neutrophils or mononuclear cells. Only three patients had a predominantly neutrophilic infiltrate (+ + +), and all three were complete responders. Two patients, one partial responder and one non-responder, also had infiltrates containing substantial numbers of neutrophils (+ +), admixed with eosinophils. Two patients, one a complete responder and the other a non-responder, had an infiltrate characterized by the absence of neutrophils.

Side-effects of treatment

Of the 17 patients who received dapsone, side-effects developed in six. Although all developed some degree of haemolysis, this was usually insignificant. However, two developed moderate haemolysis precluding any increase in dosage above 50–100 mg/day, and a further two patients developed a haemolytic anaemia necessitating a change in treatment. Sulphamethoxypyridazine was tried in both these patients and was effective and well tolerated in one, but was responsible for nausea in the other, and had to be discontinued. One patient developed headaches and malaise soon after starting on dapsone, and he was therefore changed to sulphamethoxypyridazine which was both effective and well tolerated. One patient developed a significant drop in white blood cell count (total WCC $2{\cdot}7 \times 10^9$/l, neutrophils $0{\cdot}7 \times 10^9$/l) as well as profound lethargy and nausea. The white cell count recovered rapidly when dapsone was stopped.

The single patient who received sulphapyridine (1·5 g/day) developed serious side-effects. Three weeks after starting the drug he developed a generalized drug eruption, fever and a fall in white cell count, which progressed to agranulocytosis with septicaemia, in spite of stopping the drug. With intravenous antibiotics and intensive supportive care he recovered after being seriously ill for 2 weeks.

One patient died of a myocardial infarction 1 month after starting on dapsone. This was in spite of the drug being apparently well tolerated, with no cyanosis or a significant drop in haemoglobin.

Side effects occurred in a similar proportion of responders and non-responders.

DISCUSSION

Although prednisolone and azathioprine are widely used as the standard treatment for bullous pemphigoid, the search for alternative therapies continues. The use of sulphonamides in the treatment of bullous pemphigoid was first described by Rook and Waddington[5] who reported that 11 of 17 patients treated with sulphapyridine achieved partial or total remission. However, in the pre-immunofluorescence era, no distinction between bullous pemphigoid and linear IgA disease was possible, and the latter is known to respond to sulphones and sulphonamides.[6] Person and Rogers[2] described six patients, in whom the diagnosis of bullous pemphigoid was supported immunologically, who responded to either a sulphonamide or dapsone, out of a total of 41 patients so treated. In our study of 18 patients with bullous pemphigoid, a total of seven new patients, and one patient in relapse were controlled on either dapsone or sulphamethoxypyridazine alone. A further five patients treated with dapsone and one patient treated with sulphapyridine had a partial response to treatment, but required additional or alternative drugs for full control of the disease. Our results suggest a higher response rate to sulphones or sulphonamides than the 14% response rate reported by Person and Rogers,[2] but are similar to the 45·4% response rate reported by Piamphongsant,[4] although his series included some patients with cicatricial and mucous membrane pemphigoid. In our study, 13 of the 18 patients who received either a sulphone or sulphonamide showed some response to these agents (72%), and eight patients (44%) were controlled on one of these drugs as their sole treatment.

Although in our study the majority of the patients were treated with dapsone, it seems likely that patients who respond to a sulphone will also respond to sulphonamides. This is borne out by our experience with the patient who was switched from dapsone to sulphamethoxypyridazine without control of the disease being lost.

It is of interest that neither of the patients who had been receiving long-term prednisolone responded fully to dapsone. One had been receiving tapering doses of prednisolone (maximum dose 60 mg) for 3 months, and the other had received variable doses for several years prior to the onset of bullous pemphigoid. A degree of adrenocortical suppression, sufficient to impair the normal endogenous cortocosteroid response to illness, might account for the relative resistance to dapsone treatment of these patients.

The response was less dramatic than that usually seen with dermatitis herpetiformis, but nonetheless the time to remission was relatively short. Remission was obtained in under 2 weeks in five of the complete responders, and within a month in the other two, these times being similar to those reported by Piamphongsant.[4] All the non-responders and partial responders were subsequently successfully controlled relatively easily on prednisolone (alone or in combination with dapsone or azathioprine), suggesting that their disease was not unusually aggressive or resistant to treatment. In those patients in whom prednisolone was used in combination with dapsone to induce remission, the mean dose of prednisolone required to bring the disease under control was relatively low (30 mg/day).

Piamphongsant[4] reported that older patients (aged over 60 years) with classical bullous pemphigoid with larger tense bullae showed no response to dapsone. We do not concur with this view; the mean age of the eight patients controlled on dapsone or a sulphonamide as their sole treatment was 76 years, and all of them had classical bullous lesions, while the mean age of the four non-responders was 63 years. Person and Rogers[2] noted in their series that five of the six responders had involvement of the mucous membranes, a finding which prompted further studies into the efficacy of sulpha drugs in cicatricial and mucous membrane pemphigoid, in which more than three quarters of the patients benefited.[7,8] In contrast to these findings, we are unable to identify any clinical or immunopathological features predictive of a response to a sulpha drug. The responders and non-responders were similar with respect to clinical lesion morphology, extent of disease, mucous membrane involvement and indirect IF findings. On the other hand, the nature of the inflammatory infiltrate in histological sections does appear to be of some value in predicting a response to sulpha drugs. Person and Rogers[2] reported neutrophil predominance in the dermal inflammatory infiltrate of the patients responding to sulphapyridine, and our results are in agreement with this. Only three of our patients had a striking preponderance of neutrophils in the dermal inflammatory infiltrate, and all were complete responders. However, one partial responder and one non-responder also had substantial numbers of neutrophils, as well as eosinophils and mononuclear cells in the dermal infiltrate, and of two patients in whom no neutrophils were seen, one was a responder and the other a non-responder. The nature of the inflammatory infiltrate is not, therefore, an absolute guide to the outcome of treatment with dapsone, and may, in any case, be a reflection of the age of the lesion or some other variable factor. Furthermore, treatment will often need to be commenced before histological evaluation is possible.

When effective, the drug doses required to control the disease were relatively low, the maximum being 100 mg/day dapsone and 1·5 g/day sulphapyridine. As many of the side-effects of dapsone and sulphonamides are dose related, the fact that these drugs can be effective in bullous pemphigoid at low dosage enhances their usefulness. In spite of the low dosage, we encountered a number of side effects, some of them serious, including a case of idiosyncratic agranulocytosis and drug eruption caused by sulphapyridine (1·5 g/day). The side effects encountered in the dapsone treated patients were nausea, fatigue, headache, haemolytic anaemia and neutropaenia, and one of the three patients who received sulphamethoxypyridazine also developed intractable nausea. Other reported side-effects include methaemoglobinaemia, rashes, and peripheral neuropathy.[9] In spite of the frequency of side-effects, only four of the dapsone treated patients had to discontinue treatment on account of side-effects, and three of these were subsequently tried on sulphamethoxypyridazine, which proved both effective and well tolerated in two. Although sulphones, such as dapsone, are more toxic than the sulphonamides, they are generally effective at much lower dosages and, therefore, have a more favourable therapeutic index.[9] Our experience with sulphonamides is limited, but dapsone appears to be reasonably well tolerated in bullous pemphigoid. However, as the side-effects caused by sulphones do not necessarily recur on substitution of a sulphonamide, we regard the latter as useful second line drugs in patients who have benefited from dapsone, but in whom side-effects are a problem. The toxicity of these drugs can be limited by careful monitoring of patients, particularly in the early weeks of treatment, with regular full blood and reticulocyte counts. Glucose-6-phosphate dehydrogenase deficiency should be excluded prior to starting treatment, particularly in patients of Negro or Mediterranean descent.

The mechanism of action of the sulpha drugs in bullous pemphigoid is unknown. The striking efficacy of sulphones in dermatitis herpetiformis, as well as in other diseases associated with dermal neutrophil infiltration, including subcorneal pustular dermatosis, erythema elevatum diutinum, pyoderma gangrenosum and some forms of pustular psoriasis[10] has prompted evaluation of the effect of this drug on neutrophil systems. Dapsone has been shown to suppress the generation of potentially damaging oxygen intermediates in neutrophils, particularly the hydroxyl radical[10] and to inhibit the myeloperoxidase-H_2O_2-halide system,[12] a system which is known to mediate cytotoxicity in mammalian cells.[13] In view of the possible mechanism of action on neutrophil systems, it is of interest that the three patients with the most neutrophil-rich dermal infiltrates were among the responders.

Thus, dapsone is a useful drug as a first line treatment for bullous pemphigoid, inducing remission within 2–4 weeks in 44% of patients treated using relatively low doses. Sulphonamides may also be useful in this setting, but on account of the higher doses needed and consequent higher toxicity, these agents are best reserved for dapsone-responsive patients in whom side effects are troublesome. Side-effects are relatively common, and regular and frequent blood counts are essential in the early stages of treatment. These drugs are best avoided in patients with a known history of ischaemic heart disease. Nevertheless, a trial of treatment with a sulphone, prior to resorting to corticosteroid, is worthwhile in bullous pemphigoid, particularly if there is a pre-existing contraindication to steroids such as diabetes, hypertension, peptic ulcer, obesity or osteoporosis.

REFERENCES

1 Lever WF. Pemphigus and pemphigoid; a review of the advances made since 1964. *J Am Acad Dermatol* 1979; 1: 2–31.

2 Person JR, Rogers RS. Bullous pemphigoid responding to sulphapyridine and the sulphones. *Arch Dermatol* 1977; 113: 610–15.

3 Piamphongsant T, Ausawamongkonpan S. Bullous pemphigoid controlled by dapsone. *Dermatologica* 1976; 152: 352–7.

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Vibratory angioedema: lesion induction, clinical features, laboratory and ultrastructural findings and response to therapy

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SUMMARY

We report the investigation and treatment of a 28-year-old woman with the rare condition of non-familial vibratory angiodema.

Lesions were reproduced using a vibrator set at pre-determined frequencies and amplitudes, applied for a fixed time period. There was no indication of the production of tolerance in the patient with repeated vibration, but terfenadine produced a good therapeutic response. There was no evidence of mast cell degranulation at the ultrastructural level. The patient also had mild delayed pressure urticaria and dermographism, thus demonstrating the clustering frequently found in the physical urticarias.

Vibratory angioedema is a rare type of physical skin reactivity in which vibration causes the development of localized erythema and oedema. Two families have been described in which the condition showed autosomal dominant inheritance, and the gene has a high penetrance.[1,2] There is also a report of non-familial occurrence[3] and one of occupationally acquired vibratory angioedema.[4] Histamine may play a major role in the development of lesions[5] although to date the effect of antihistamines has not been evaluated. Atopy has not been found to be more frequent in these patients or their families and there is no known immunological basis for the reaction.

CASE REPORT

The patient, a 28-year-old woman, had suffered, since the age of 18, from itching followed by erythema in areas of skin which had been subjected to vibration.

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Vibration induced by clapping the hands for five minutes, and/or use of a power lawn mower or garden shears, produced tingling, itching and erythema affecting the wrists and forearms within minutes. However, her main problems occured when walking or jogging. Less than 10 min walking produced itching followed by erythema on the anterior thighs. If she stopped, the itching disappeared, but if walking was continued, the itching became more severe and facial flushing, chest tightness and an associated generalized feeling of heat developed. The systemic symptoms disappeared within minutes of resting. On recommencing walking the rash reappeared within 10 min. Jogging for 5 min produced itching and a blotchy erythema on the thighs and abdomen followed by generalized itching on the body and facial flushing, progressing to widespread flushing and chest tightness.

Additionally, she found that pruritic erythematous lesions developed at localized sites of pressure produced by tight jeans, a tight waist band or sitting on a hard edged chair. There was no personal or family history of atopy or physically induced angioedema or urticaria.

INVESTIGATIONS

The following investigations were carried out on separate occasions.

Laboratory investigations

Haemoglobin, white cell count and erythocyte sedimentation rate were normal and were not altered by the production of lesions. The serum IgE level was normal (38 IU/ml). Protease inhibitors and complement levels were normal both before and after lesion induction.

Clinical investigations

Vibration challenge testing. Preliminary testing by resting the forearm gently on a laboratory Whirlmixer (WM/250SC/P—Jencons, Hemel Hempstead, Hertfordshire, U.K.) for 5 min produced swelling in the forearm which could not be produced in a patient with symptomatic dermographism or in a normal control subject.

For quantitative studies, a calibrated vibrator was set up consisting of a Derritron vibrator (VP4, Derritron Ultrasonics Ltd., Hastings, East Sussex U.K.) attached to a Derritron power amplifier (100W, Derritron Ultrasonics Ltd.) and a Phillips vibration meter (PR952/93—A.W. Philips Ltd., London U.K.).

The patient's right and left forearm were measured at marked sites at the wrist, mid forearm and proximal forearm distal to the elbow joint. The vibrator was initially set at a frequency of 12 cycles/s and an amplitude of 1 mm. The patient grasped the vibrator bar using the right hand, which was vibrated for 7 min. Using the left hand, the vibration was increased to a frequency of 13 cycles/s at an amplitude of 1 mm for 10 min. The clinical response and change in circumference at the marked sites were recorded. To demonstrate an area of localized angiodema the position of the left hand was changed and the left arm vibrated using an amplitude of 2 mm and a frequency of 13 cycles/s for 10 min.

Challenge testing for other physical urticarias. A calibrated dermographometer was used to test for symptomatic dermographism at pressures of 2·0, 5·87, 9·75 and 14·12 × 10^5 Pa (20·4, 59·9, 99·4 and 144·0 g/mm^2) on the upper back,[6] and to test for delayed pressure urticaria at a pressure of 9·75 × 10^5 Pa (99·4 g/mm^2) applied for 5, 15, 33, 70 and 100 s.[7] Pressure application was performed on the back lateral to the spine from above downwards. Weal and flare reactions were assessed after 5 min and pressure test sites were examined after 2 and 6 h. Testing in a hot bath at

40°C for 10 min and ice cube testing, for cholinergic and cold urticaria, respectively, were also carried out.

Histological, immunological and ultrastructural studies

A control 4 mm punch biopsy, using 2% lignocaine as local anaesthetic, was taken from the skin of the medial aspect of the left forearm. A repeat 4 mm punch biopsy was taken from the same area following the production of a localized area of erythema and angiodema by vibratory stimulation of 13 cycles/s and 2 mm amplitude for 10 min. The biopsy was taken 2 min after cessation of vibration. The tissue was divided into portions for routine histopatholgy, immunofluorescence and electron microscopy. For electron microscopy, the specimen was fixed in half-strength buffered 0·04 M Karnovsky fixative, washed in 0·06 M Cacodylate buffer, post-fixed in 2% osmium tetroxide, dehydrated in graded ethanols, and embedded in Epon. Thin sections were stained on a grid with uranyl acetate and lead citrate. The epidermis and the full thickness of the dermis were examined.

Therapy

Lesions were induced using the vibrator set at a frequency of 13 cycles/s and an amplitude of 2 mm for 10 min. Terfenadine 120 mg was given orally and an identical vibratory stimulus applied 2 h later.

RESULTS

Clinical

Vibration challenge at an amplitude of 1 mm and frequency of 12–13 cycles/s for 7–10 min produced tingling of both forearms at 2 min. Itching and a blotchy macular erythema became progressively more severe from 4 min, and on cessation of vibration both mid forearms appeared oedematous. There was an increase in mid forearm circumference on both sides and the left side also showed an increase at the wrist and proximal forearm (Table 1).

The oedema was diffuse, but there was a localized area of erythematous subcutaneous swelling on the superior aspect of the forearm where vibration was felt to be maximal by the patient.

Following a single oral dose of 120 mg terfenadine tingling was considerably reduced and delayed from 2 min to 7 min and erythema was barely perceptible at 9 min.

TABLE 1. Effect of vibration on forearm circumference

	Right			Left		
	Wrist	Mid forearm	Proximal forearm	Wrist	Mid forearm	Proximal forearm
Circumference prior to vibration (cm)	15·7	21·6	24·7	15·7	21·3	24·3
Circumference after vibration (cm)	15·7	22·0	24·7	16·2	21·7	24·7

Left arm vibration: 12 cycles/s, 1 mm amplitude for 7 min; Right arm vibration: 13 cycles/s, 1 mm amplitude for 10 min.

Jogging slowly on the spot for 10 min produced itching on the abdomen at 90 s. As jogging proceeded itching became progressively more severe and was followed by a feeling of heat in the skin and flushing across the chest. A blotchy macular erythema appeared on the abdomen at 5 min. This was maximal at 10–12 min and had disappeared completely by 90 min. In order to exclude a cholinergic mechanism, a 6% aqueous solution of hyoscine hydrobromide was applied to a 10 cm^2 area on the right lower abdomen, and left in contract for 5 min. On repeat jogging the reaction was not blocked, although sweating was blocked. Mild dermographism was found, i.e. production of a weal and flare at 5 min by stroking the skin using a pressure $5{\cdot}87 \times 10^5$ Pa/mm^2, but this stimulus did not cause itching. A papule was present at each pressure test site at 2 h, confirming mild delayed pressure urticaria. This had disappeared by 6 h. Challenge tests for cholinergic and cold urticaria were negative.

No pathological changes were detected on routine histopathological examination and mast cell numbers and morphology appeared normal using the choloracetate esterase reaction.

No specific changes were seen on direct immunofluoresence. Both control and lesional specimens showed patchy granular deposition of IgM in the basement membrane zone with some colloid bodies.

Electron microscopy

Non-lesional skin exhibited no ultrastructural abnormalities apart from the presence of large vacuoles within the cytoplasm of keratinocytes in the basal layer of the epidermis where they were apposed to melanocytes and also within some of the melanocytes. A similar feature was seen in lesional skin (Fig. 1). In both control and lesional skin, mast cells were much more frequently encountered than is usual in healthy skin, especially near vessels (Fig. 2), but we did not attempt to count the mast cells. The granules were of normal morphology, and there was no evidence of degranulation either inside or outside the mast cells in lesional skin (Fig. 3).

The lumina of arterioles, venules, and capillaries in the lesional skin were more dilated than

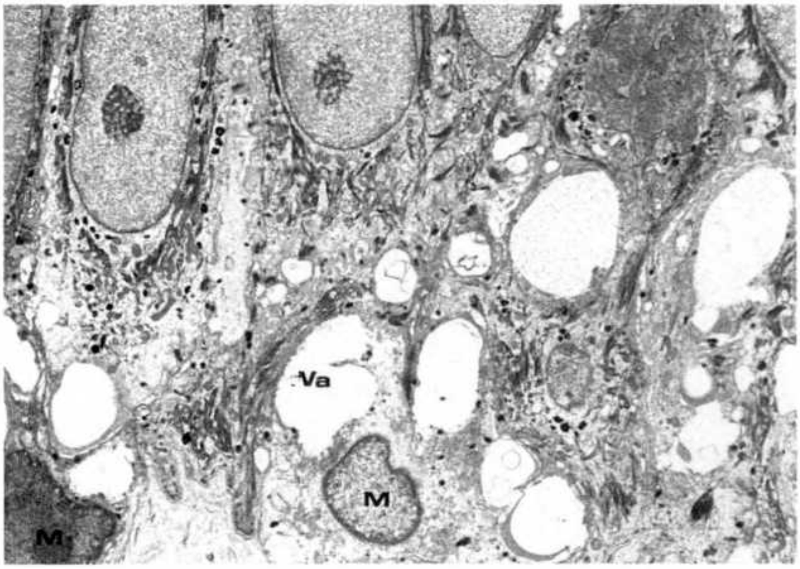

FIGURE 1. Vacuoles (Va) in cytoplasm of basal keratinocytes apposed to melanocytes (M) in lesional epidermis. (Original × 5000.)

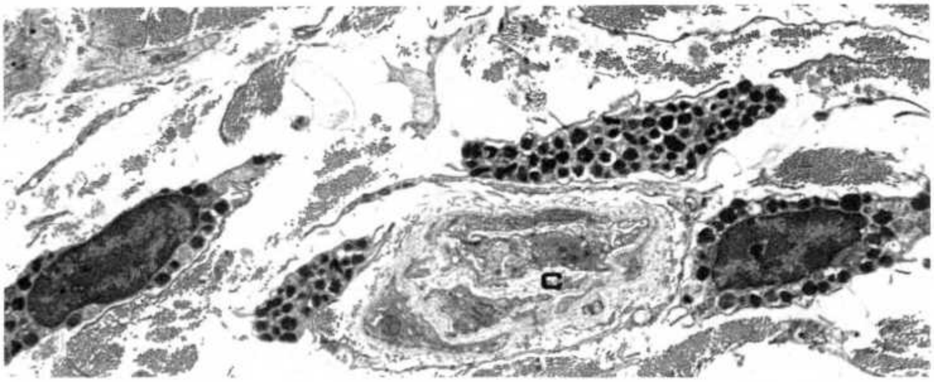

FIGURE 2. Four mast cells adjacent to an arteriolar capillary (C) in lesional skin. There is no evidence of degranulation. (Original × 4600.)

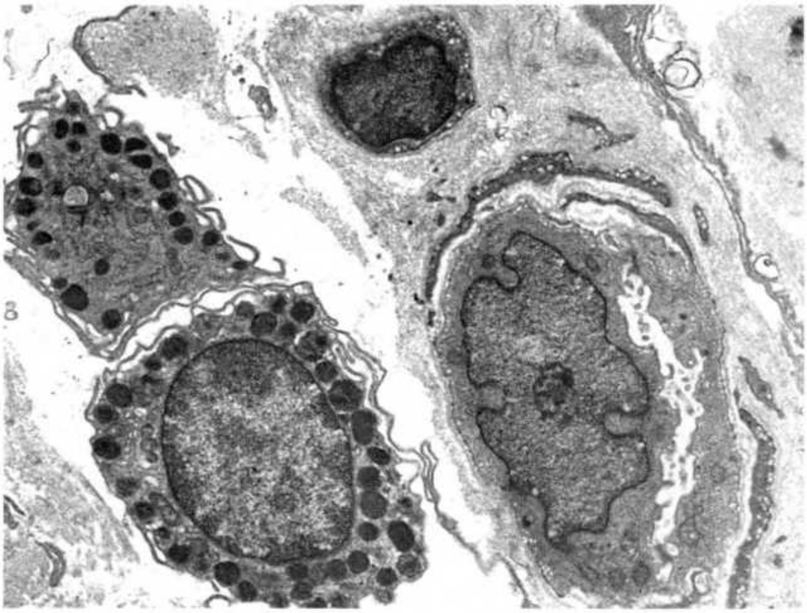

FIGURE 3. Mast cells adjacent to arteriolar capillary in lesional skin. The granules are of normal morphology, and there is no evidence of degranulation. (Original × 7500.)

those in control skin, but appositional junctions between endothelial cells were intact, and their general ultrastructural morphology was normal (Fig. 4). Terminal neurites close to vessels also appeared normal. Neutrophils were not seen, and the general cell population of the dermis, and the appearance of the matrix and collagen bundles seemed normal.

DISCUSSION

Vibratory angioedema was first described by Patterson *et al.*[1] in a Swedish family, and

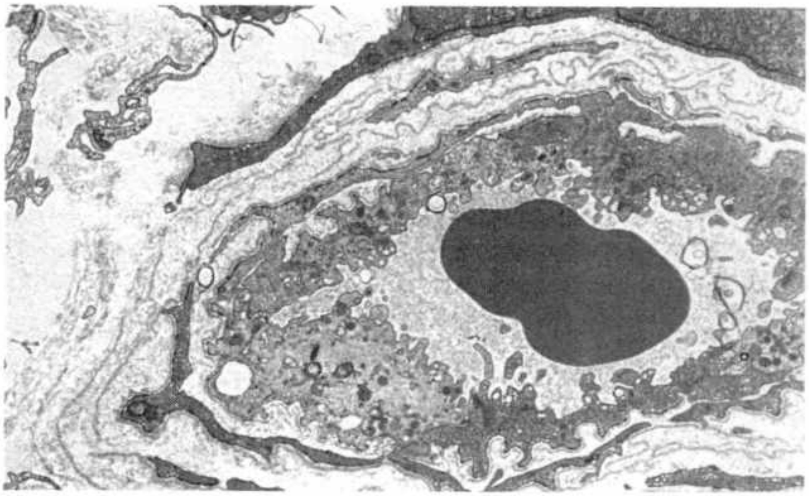

FIGURE 4. A venule in lesional skin. The lumen is dilated and contains an erythrocyte. Cytoplasmic organelles of the endothelial cells appear normal and appositional contacts are intact. (Original × 7500.)

inheritance was autosomal dominant with high penetrance. Epstein and Kidd[2] described an identical disorder in a family of Christian Lebanese origin. In both families, showering, towelling and massaging the skin provoked symptoms and the disorder was noted shortly after birth. Onset of symptoms and signs occurred within minutes of vibration and could last for several hours. Epstein and Kidd[2] suggested that an induction of tolerance is possible if patients 'warm up' gradually and one patient has successfully become tolerant.[3] However, during our investigations there was no evidence of the development of tolerance and neither was this reported in the original cases.[1] A sporadic (non-inherited) case of this disorder has been reported[3] and an occupationally acquired form has been found in a metal grinder.[4] Our patient is another sporadic case and the first in whom laboratory measurements have been made of the stimulus productng the lesions in terms of frequency, amplitude and time of exposure. We tested vibration at 10, 12, 13, and 16 cycles/s and found that 13 cycles/s was the most effective in producing lesions. An amplitude of 2 mm, the maximum our equipment could produce, was more effective than 1 mm amplitude.

Metzger *et al.*[5] demonstrated an increase in circulating plasma histamine, peaking within 5 min and resolving within 15 min, in vibratory angioedema, which suggests that histamine may play a major role in the development of this reaction. At an ultrastructural level Ting *et al.*[3] reported mast cell degranulation following vibratory challenge, but this was not observed on electron microscopic examination of a large number of cells in lesional skin of our case. Routine histopathology and the chloroacetate esterase reaction revealed normal numbers of mast cells, but on electron microscopy they appeared to be more numerous than normal in both uninvolved and lesional skin. This impression might have been confirmed if the cells had been counted on light microscopy. It is possible that the rapid release of histamine into the circulation may occur from endothelial cells or from another non-mast cell source.

At the ultrastructural level, the large vacuoles within the cytoplasm of keratinocytes in the basal layer have not been observed previously in vibratory angioedema, but have been found in cold urticarial weals and in histamine induced weals in normal subjects[8] indicating that oedema is present. In our control skin biopsy this phenomenon may have been the result of previous clinical or possibly sub-clinical wealing.

The response to terfenadine indicated by delayed onset of symptoms in the laboratory reflects the good clinical response reported by the patient. She found that she could walk for over an hour if she took the drug prophylactically. Our patient also had mild dermographism and mild delayed pressure urticara. Clustering of physical urticarias in the same patient is well known, although it is surprising that this combination of immediate and delayed reactions occurred, suggesting different pathological reactions to physical stimuli.

ACKNOWLEDGMENTS

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REFERENCES

1 Patterson R, Mellies CJ, Blankenship ML, Pruzansky JJ. Vibratory angioedema: A hereditary type of physical hypersensitivity. *J Allergy Clin Immunol* 1972; **50:** 184–2.

2 Epstein PA, Kidd KK. Dermo-distortive urticaria. An autosomal dominant dermatologic disorder: *Am J Med Genet* 1981; **9;** 307–15.

3 Ting S, Reimann BEF, Rer Nat *et al.* Non-familial, vibration-induced angioedema. *J Allergy Clin Immunol* 1983 **71:** 546–51.

4 Wener MH, Metzger WJ, Simon RA. Occupationally acquired vibratory angioedema with secondary carpal tunnel syndrome. *Ann Int Med* 1983; **98:** 44–6.

5 Metzger WJ, Kaplan AP, Beaven MA. *et al.* Hereditary vibratory angioedema: Confirmation of histamine release in a type of physical hypersensitivity. *J Allergy Clin Immunol* 1976; **57:** 605–8.

6 Breathnach SM, Allen R, Milford Ward A, Greaves MW. Symptomatic dermatographism; clinical features, laboratory investigations and response to therapy. *Clin Exp Dermatol* **8:** 463–76.

7 Estes SA, Yung CW. Delayed pressure urticaria: An investigation of some parameters of lesion induction. *J Am Acad Dermatol* 1981; **5:** 25–31.

8 Eady RAJ, James MP, Kennedy AR. Epidermal changes occurring in histamine weals and cold urticaria. *Br. J Dermatol* 1981; **105:** 348.

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Chronic leg ulceration with livedoid vasculitis, and response to oral ketanserin

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SUMMARY

We report a patient with a 6-year history of recalcitrant painful ulceration of both lower legs, diagnosed as being due to livedoid vasculitis. The lesions healed rapidly and remained healed on treatment with oral ketanserin.

CASE REPORT

A 51-year-old Caucasian woman had suffered from recurrent painful ulceration of both lower legs for 6 years. Although some lesions had healed after many months leaving white atrophic areas, the patient had not been free of lesions at any time during this period. For 20 years she had suffered from Raynaud's phenomenon affecting her hands and feet, and chilblains during the winter months. On examination there were areas of livedo reticularis on the thighs and knees and several well demarcated ulcers (Fig. 1a) on the extensor and medial surfaces of both feet with areas of atrophie blanche at sites of healed lesions. Investigations showed a normal full blood count, ESR, coagulation screen and platelet aggregation studies. ANA was positive at a titre of 1:40 with a speckled pattern and anti-DNA antibodies were not detected. Doppler studies of the dorsalis pedis and posterior tibial arteries were normal.

Histology

A skin biopsy from the edge of an ulcer showed incipient epidermal ulceration and necrosis in association with endothelial cell swelling, thrombosis and occlusion of adjacent blood vessels (Fig. 2). Some of the blood vessels in the reticular dermis showed fibrinoid changes and hyaline thickening of their walls. There also was evidence of recanalization of some vessel lumina, extravasation of red blood cells and a minimal perivascular lymphocytic infiltrate beneath the ulcerated surface. These findings were compatible with livedoid vasculitis.

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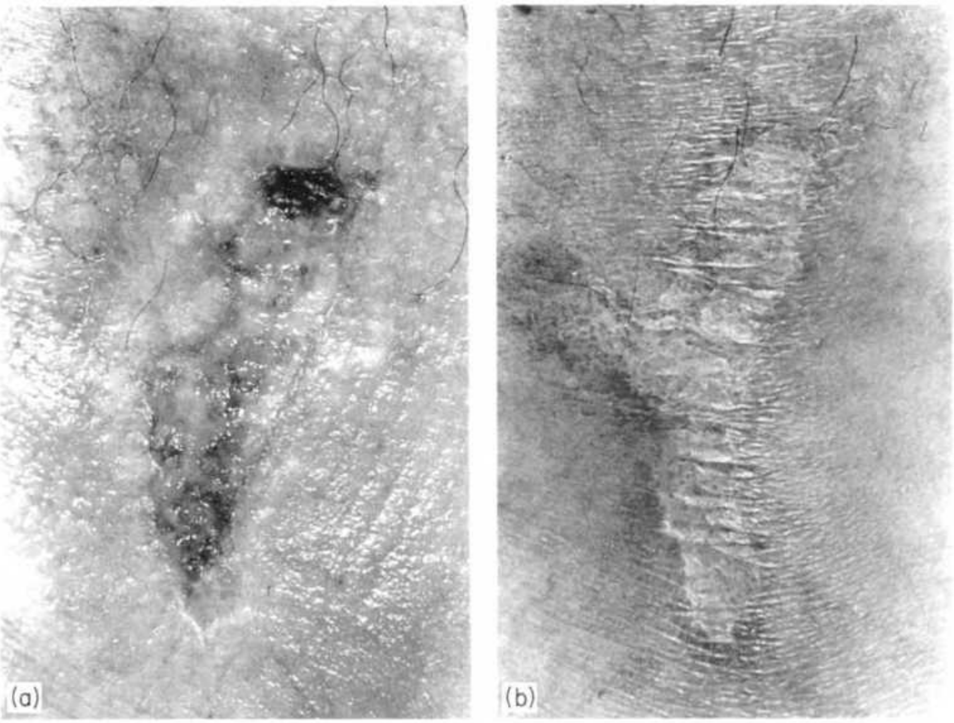

FIGURE 1. Ulcerated area on lateral aspect of right shin (a) before treatment and (b) following treatment with ketanserin 20 mg daily for 6 weeks. The lesion has healed leaving ivory-white scarring.

Treatment

There was no response to various topical treatments and in view of her symptomatic Raynaud's phenomenon the patient was started on nifedipine retard 60 mg daily. New ulcers continued to develop, and no benefit was observed following treatment with diltiazem 60 mg twice daily or captopril 12·5 mg twice daily, each taken for 2 months. A therapeutic trial with ketanserin 20 mg twice daily was commenced in November 1987 and within 6 weeks all the ulcers had healed (Fig. 1b). At the time of writing the patient had been on continuous treatment for 9 months and no new lesions had developed during this period. Furthermore during treatment with ketanserin the patient spontaneously volunteered that her attacks of Raynaud's phenomenon were less frequent and less severe.

DISCUSSION

Livedoid vasculitis is an occlusive vascular disease with segmental hyalinization involving the middle and lower parts of the dermis in the absence of polymorphonuclear leukocyte infiltration and leukocytoclasis.[1] Clinically, it presents with painful often recurrent slowly healing ulceration of the legs and ankles, together with areas of atrophie blanche which are stellate ivory-white atrophic plaques with speckled telangiectasia and surrounding hyperpigmentation. Atrophie blanche may be considered as a final scarring process of the skin and Ryan[2] lists many

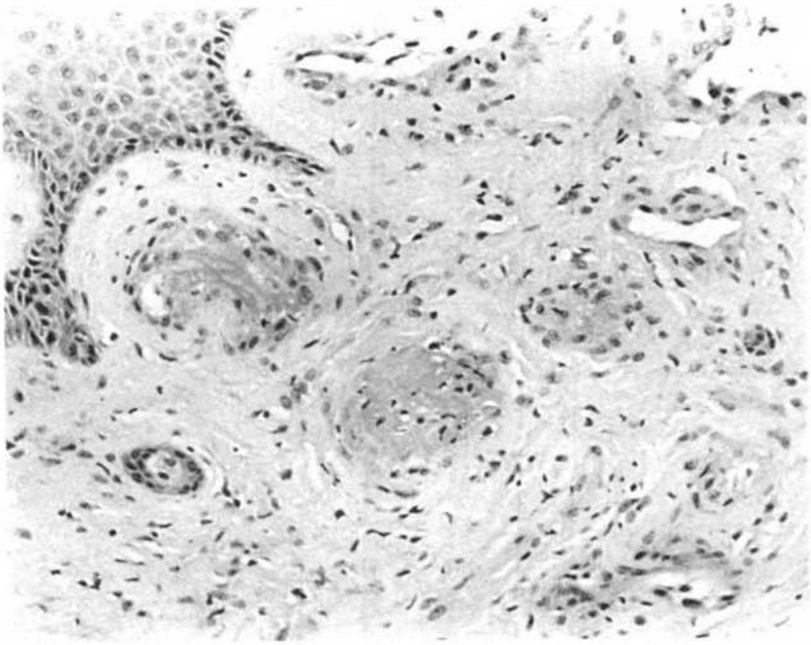

FIGURE 2. Skin biopsy from the edge of an ulcer showing thrombosis, and occlusion of a blood vessel with minimal perisvascular inflammatory cell infiltration (original × 80).

diseases with which it is associated, the most common being venous stasis. Gray *et al.*[3] emphasized that any clinical similarity between atrophie blanche and the atrophic scars on the lower extremities of patients with chronic venous stasis can be separated by the vasculitic pathology of the former, and these authors stated that the two diseases are completely separate entities. Our patient had no evidence of chronic venous stasis, judged both clinically and by Doppler studies. Livedoid vasculitis may occur by itself, be associated with livedo reticularis or accompany a connective tissue disease.[4] It is not clear from the literature whether the patients described with livedo reticularis and summer ulceration[5] should be included in the diagnosis of livedoid vasculitis as suggested by Winklemann[4] or whether they represent an overlap with an associated disease such as systemic lupus erythematosus or polyarteritis nodosa.

The fact that drugs which interfere with platelet function[6,7] (aspirin, dipyridamole and ticlopidine) or enhance fibrinolytic activity[8] (phenformin and ethylestrenol) are reported to be efficacious in the treatment of livedoid vasculitis suggests that the pathogenesis might involve a coagulopathy. This might involve a disturbance in the haemostatic function of the endothelial cells or an alteration in platelet function or both.

Serotonin, a vasoactive factor released from the dense granules of activated platelets possesses both vasoconstrictor and vasodilator properties. It causes contraction of vascular smooth muscle through activation of S_2-serotonergic receptors,[9] an augmentation of the action of other vacoconstrictor agonists such as angiotensin II and noradrenaline[10] and an increase in the release of noradrenaline from adrenergic nerves.[11] Alternatively, through activation of S_1-serotonergic receptors, serotonin can cause vasodilation by stimulating the release of endothelium-derived relaxing factor.[12] It was on the basis of the theoretical possibility that an

S_2-serotonergic receptor antagonist might prevent the vasoconstrictor action of serotonin and increase cutaneous blood flow that ketanserin was administered to our patient.

Ketanserin 3-[2-[4-(*p*-fluorobenzoyl)piperidino]ethyl]-2,4(1H,3H)-quinazolinedione is the first selective S_2-serotonergic blocking agent which is devoid of agonistic properties.[13] In clinical studies, ketanserin has been shown to increase the digital blood flow in patients with Raynaud's phenomenon and reduce the frequency of attacks.[14] It also has been shown to lower blood pressure in patients with essential hypertension without causing reflex tachycardia or orthostatic hypotension, and a dose of 80 mg daily was found to be as effective as metoprolol 200 mg daily.[15] There are conflicting reports about the effect of ketanserin on the symptoms of intermittent claudication,[16] but the drug has been shown to improve respiratory function in patients with chronic obstructive airways disease[17] and to reduce the frequency and severity of flushing in patients with carcinoid syndrome.[18]

Ketanserin is rapidly absorbed following oral administration with peak levels being achieved within 0·5 to 2 h.[13] It has a half-life of about 16 h, and distribution studies have shown that 10% exists as free ketanserin, 27% is bound to red blood cells and 63% is bound to plasma proteins. The drug is extensively metabolized, with ketone reduction and oxidative N-dealkylation in the liver (the metabolites not contributing to the overall pharmacological activity of ketanserin) and excretion is predominantly in the urine. The therapeutic dose of ketanserin is 20–40 mg twice daily and this is usually well tolerated. Side-effects including drowsiness, lack of concentration and lightheadedness are sometimes reported during the first days of treatment 1–2 h after taking the drug but these effects are dose-related and subside after a few days of treatment. There have been reports that ketanserin has the potential to lengthen the QT_c interval of the electrocardiogram.[19] Because the propensity to produce arrhythmias is increased by hypokalaemia, severe bradycardia and certain anti-arrhythmic drugs which further prolong repolarization, caution is required in administering ketanserin in such circumstances.[20]

The rapid relief of pain and healing of the ulcers in our patient was not thought to have been due to a spontaneous remission of the disease since the livedoid vasculitis was of long standing and other drug therapy had been unsuccessful. Ketanserin might have produced its beneficial effect through several mechanisms. Firstly, serotonin released from aggregating platelets exerts a positive feedback effect on other platelets to produce secondary recruitment and ketanserin inhibits this serotonergic amplification reaction.[21] Secondly, ketanserin might have increased cutaneous blood flow by inhibiting serotonin-induced smooth muscle constriction mediated by S_2-receptors and through its α_1-adrenoceptor antagonist actions.[22] Furthermore, ketanserin has been shown to increase red cell deformability and reduce the 'stickiness' of white blood cells,[23,24] both of which are important determinants of blood flow in the microvasculature. In addition, ketanserin itself may have a beneficial effect on wound healing since topical application promotes granulation tissue formation and re-epithelialization of decubitus ulcers.[25] We believe that the benefit observed in our patient warrants further study of ketanserin in the treatment of livedoid vasculitis.

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REFERENCES

1 Bard JW, Winkelmann RK. Livedo vasculitis: segmental hyalinizing vasculitis of the dermis. *Arch Dermatol* 1967; **96:** 489–99.

2 Ryan TJ. Livedoid vasculitis, segmental hyalinising vasculitis, atrophie blanche, livedo with summer or winter ulceration. In: *Microvascular Injury*. London: Saunders, 1976: 330–2.

3 Gray HR, Graham JH, Johnson W, Burgoon CF. Atrophie blanche: periodic painful ulcers of lower extremities. *Arch Dermatol* 1966; **93:** 187–93.

4 Winkelmann RK, Schroeter AL, Kierland RR, Ryan TM. Clinical studies of livedoid vasculitis (segmental hyalinizing vasculitis). *Mayo Clin Proc* 1974; **49:** 746–50.

5 Feldaker M, Hines S, Kierland EA. Livedo reticularis with summer ulcerations. *Arch Dermatol* 1955; **72:** 31–42.

6 Drucker CR, Duncan WC. Antiplatelet therapy in atrophie blanche and livedo vasculitis. *J Am Acad Dermatol* 1982; **7:** 359–63.

7 Yamamoto M, Danno K, Shio H, Imamura S. Antithrombotic treatment in livedo vasculits. *J Am Acad Dermatol* 1988; **18:** 57–62.

8 Gilliam JN, Herndon JH, Prystowsky SD. Fibrinolytic therapy for vasculitis of atrophie blanche. *Arch Dermatol* 1974; **109:** 664–7.

9 Vanhoutte PM. Serotonin and the vascular wall. *Int J Cardiol* 1987; **14:** 189–203.

10 Van Nueten JM, Janssens WJ, Vanhoutte PM. Serotonin and vascular smooth muscle. In: *Serotonin and the Cardiovascular System* (Vanhoutte PM, ed). New York: Raven Press, 1985; 95–103.

11 McGrath MA. 5-hydroxytryptamine and neurotransmitter release in canine blood vessels: inhibition by low and augmentation by high concentrations *Circ Res* 1977; **41:** 428–35.

12 Vanhoutte PM. Platelets, endothelium and vasospasm. *Thromb Haemostas* 1987; **58:** 252.

13 Van Nueten JM, Janssen PAJ, Symoens J *et al.* Ketanserin. In: *New Cardiovascular Drugs* (Scriabine AF ed). New York: Raven Press, 1987; 1–56

14 Meloni F, Transi MG, Sciacca V *et al* Therapeutic efficacy of ketanserin, a selective antagonist of the serotonin (5-HT$_2$) receptors, in primary and secondary Raynaud's phenomenon. *Angiology* 1987; **38:** 530–6.

15 Sheps SG, Schirger A, Zachariah PK *et al.* Comparison of ketanserin and metoprolol in the treatment of essential hypertension. *Arch Intern Med* 1987; **147:** 291–6.

16 Cameron HA, Waller PC, Ramsay LE. Placebo-contolled trial of ketanserin in the treatment of intermittent claudication. *Angiology* 1987; **38:** 549–55.

17 Cazzola M, D'Amato G, Lobefalo G *et al.* Ketanserin, a new blocking agent of serotonin S$_2$-receptors; respiratory functional effects in chronic obstruction of the airways. *Chest* 1987; **92,** 863–6.

18 Gustafsen J, Lendorf A, Raskov H, Boesby S. Ketanserin versus placebo in carcinoid syndrome. *Scand J Gastroenterol* 1986; **21:** 816–18.

19 Bazan A, Aladariz AE, Baglivo H *et al.* QT-prolongation by ketanserin and induction of malignant ventricluar arrythmias. *Cardiologica* 1985; **5:** 588–92.

20 Singh BN, Nademanee K, Symoens J, Janssens M. Ketanserin and QT$_c$ prolongation. *Eur Heart J* 1987; **8:** 667–8.

21 Holmsen H. Platelet activation and serotonin. In. *Serotonin and the Cardiovascular System* (Vanhoutte PM, ed). New York. Raven Press, 1985; 75–86

22 Leysen JE, Awouters F, Kennis L *et al.* Receptor binding profile of R 41 468, a novel antagonist at 5-HT$_2$ receptors. *Life Sci* 1981; **28:** 1015–22.

23 Zannad F, Voisin Ph, Pointel JP *et al.* Effects of ketanserin on platelet function and red cell filterability in hypertension and peripheral vascular diseases. *J Cardiovasc Pharmacol* 1985; **7:** S32–S34.

24 Walker RT, Matrai A, Bogar L, Dormandy JA. Serotonin and the flow properties of blood. *J Cardiovasc Phamacol* 1985; **7:** S35–S37.

25 Internal Clinical Research Report, Janssen Pharmaceutica, Beerse, Belgium, 1988.

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Acute acral pustulosis

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SUMMARY

We report a patient with a pustular eruption in an acral distibution that developed abruptly 4 days after a streptococcal pharyngitis. The histology showed an intraepidermal vesicle filled with neutrophils with an underlying lymphocytic vasculitis. Direct immunofluorescence demonstrated heavy deposition of C_3 within the vessel walls. In addition, circulating immune complexes were found.

An acute pustular eruption preceded by an infection, usually of the upper respiratory tract, has been described by several authors.[1-4] The onset is abrupt and the eruption is distributed mainly on the hands and feet but may extend to involve the entire body. Biopsy of the lesions has shown a vasculitis of the upper dermal vessels with deposition of immunoreactants.[2,3] Resolution takes place over 2–3 weeks, and there appear to be no sequelae. This constellation of findings is felt to be consistent with a true bacterid. We report here a patient with an eruption of this type with the additional finding of circulating immune complexes.

CASE REPORT

A 32-year-old black male patient presented to the Dermatology Clinic of the Veterans Administration Hospital with numerous pustules distributed over the hands, feet, forearms, and lower legs. He stated that the eruption had arisen 'overnight' 4 days previously and had changed very little since that time. He had neither a personal nor family history of dermatological disease nor any other significant medical disorder. He was taking no medications. Four days prior to the onset of the pustular eruption, he had been seen in the emergency room of the same hospital for a sore throat, and a throat swab grew a beta-haemolytic streptococcus Group A. He was given a single intramuscular injection of benzathine penicillin. The patient had taken penicillin orally several times in the past for other infections without untoward reactions

On examination there were multiple, symmetrically distributed pustules and a few vesicles on the palms and soles (Fig. 1). Some of the pustules extended onto the dorsal surfaces of the hands

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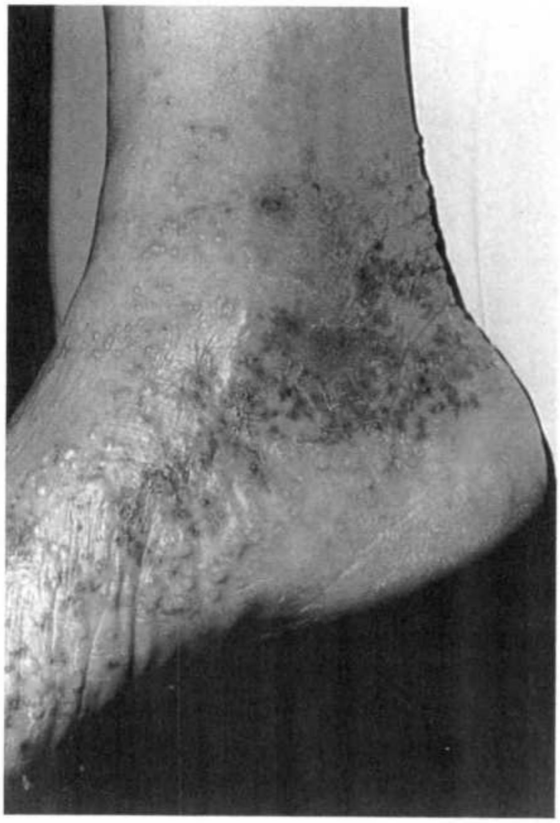

FIGURE 1. Multiple haemorrhagic pustules and vesicles on the sole extending onto the dorsum of the foot.

and feet. There were a few scattered pustules on the forearms as well as on the lower legs and knees. The pustules ranged from several millimeters to over 1 cm in diameter. Most of them were surrounded by a ring of erythema and purpura. Some purpuric macules without pustules or vesicles were also seen. No pitting of the nails was seen and the remainder of his skin and his oral mucosa were normal.

Laboratory investigations were normal, but the antistreptolysin O titre was elevated to 340 Todd Units (normal: 0–50 Todd Units). Potassium hydroxide examination as well as skin cultures for bacteria and fungi were negative. Circulating immune complexes were markedly elevated to 220 μg AHG equivalents/ml (normal < 50 μg AHG equivalents/ml) using the Raji assay technique. Skin biopsy revealed an intra-epidermal pustule filled with neutrophils. Immediately below the pustule, there was a dense perivascular infiltrate of lymphoid cells with a smaller number of neutrophils (Fig. 2). On direct immunofluorescence, there was a marked deposition of fibrin in the upper dermal vessels. Direct immunofluorescence for IgG, IgM, and IgA was negative, but showed heavy granular deposits of C_3 in the vessel walls. Electron microscopy confirmed the heavy fibrin deposition and demonstrated damage to the upper dermal vessels.

The eruption resolved completely over the following week. The patient was given topical Cyclocort® (0·1% amcinonide) ointment to apply three times a day, but he stated that the

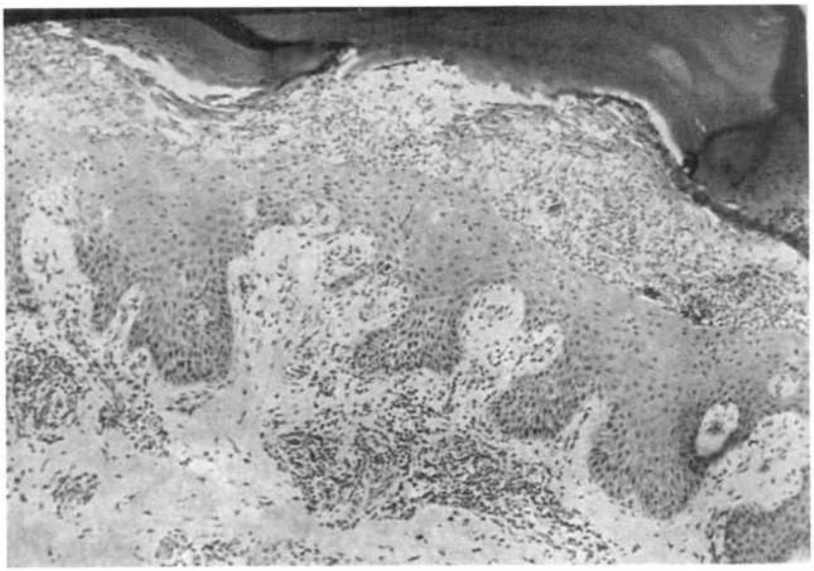

FIGURE 2. Intra-epidermal pustule filled with neutrophils. Below this there is a dense perivascular infiltrate of lymphoid cells with a smaller number of neutrophils. (Haematoxylin and eosin, original × 10.)

eruption showed noticeable improvement several days prior to his actually beginning the medication (which was delayed in the post). There was no recurrence, nor did the patient develop any other dermatological condition over a 1-year folow-up period, except tinea cruris.

DISCUSSION

In the early nineteen-thirties Andrews *et al.*[5,6] used the term pustular bacterid to describe some recalcitrant pustular eruptions of the palms and soles thought to be caused by distant foci of infection. This suggestion of a relationship of chronic palmoplantar pustulosis to infection was not entirely well received, however.[7,8] Stevens and Ackerman[9] concluded, in a recent review of the literature on the subject, that the majority of the cases represented either a localized pustular psoriasis or a dermatitis. Most physicians agree that treatment of an infectious focus will offer little benefit in this chronic form of palmoplantar eruption. In most cases such a focus is probably unrelated.[4]

The acute form of this pustular eruption, on the other hand, may indeed be related to a preceding infection. Baker and Ryan[1] reported five patients with no history of prior skin disease who developed an 'exanthematic' form of pustular psoriasis preceded by a sore throat. The rash usually began on the hands and feet and rapidly spread to the rest of the body. The authors admitted that it was unclear whether this represented true psoriasis or a psoriasiform reaction to some unknown stimulus.

Hellgren and Mobacken[10] expressed the belief that an infectious focus in the body, such as an acute upper respiratory infection, can initiate a sterile, pustular eruption. Baker and Wilkinson[11] also felt that there was a realtionship between an acute pustular eruption and a preceding infection.

Tan[2] reported a case of disseminated pustules with accentuation on the distal extremities following a streptococcal pharyngitis. An intra-epidermal pustule filled with neutrophils with an underlying leukocytoclastic vasculitis was seen on histological examination. Tan[2] proposed a non-allergic mechanism in which streptococcal products are disseminated into the blood stream and deposited in dermal vessels inducing a direct toxic effect. The damaged vessels then attract neutrophils by a chemotactic mechanism. Neutralizing antibodies against this antigen are also produced. He believed that this condition was rare because only a few types of streptococci elaborate 'skin toxins' just as only certain types produce erythrogenic toxin or are nephrotoxic.

Other investigators[3] have noted similar clinical presentations with a histology of a leukocytoclastic vasculitis and with fibrin deposition in the vessel walls. In addition, on direct immunofluorescence, deposits of C_3 were seen in the vessels in all the cases as well as deposits of IgM in some of the cases.

Burge and Ryan[4] reported five cases of an acute pustular eruption precipitiated by a preceeding febrile illness. They felt that these cases may represent examples of true bacterid reactions. Three of the patients had no previous history of dermatological disease nor a family history of psoriasis. However, one patient had a history of psoriasis and one had chronic palmoplantar pustulosis. Features that most of these cases and the previously reported cases share, include, as outlined by Burge and Ryan:[4] (1) a preceding infection of the upper respiratory tract, (2) an abrupt onset over 24–48 h, (3) many pustules on the palmoplantar surfaces that may extend to the dorsal surfaces of the hands and feet or the entire body, (4) discrete unilocular pustules measuring up to 1 cm, and (5) rapid resolution over 2–3 weeks. Tan[2] and Ishakawa *et al.*[3] commented on the additional findings of leukocytoclastic vascultitis and immune deposits in the vessels of the upper dermis.

Our patient demonstrated all the characteristic features outlined by Burge and Ryan.[4] In addition, deposition of C_3 in the vessel walls was seen on direct immunofluorescence as in the cases described by Ishakawa *et al.*[3] To our knowledge, our patient is the first reported case in the British literature to show circulating immune complexes. Also unique to our case is the demonstration of a lymphocytic vasculitis instead of an obvious leukocytoclastic vascultitis. This is unusual, as one would expect the inflammatory infiltrate to consist largely of neutrophils in a necrotizing vasculitis induced by immune complexes that have activated the complement system.[12] Nevertheless, a predominance of infiltrating lymphocytes with few neutrophils in cases of cutaneous necrotizing vasculitis has been reported repeatedly.[13–15] Immune complexes have been shown to activate lymphocytes to produce lymphokines which may be chemotactic or cytolytic.[16]

The possibility that our patient's pustular eruption could represent a drug reaction to penicillin was also considered. Macmillin[17] attributed four cases of generalized pustular eruptions to drugs. Biopsy revealed a dermal lymphohistiocytic infiltrate in all cases. At least two other patients with generalized pustular eruptions secondary to a drug have been reported with histological evidence of a leukocytoclastic vasculitis.[18] However, the fact that our patient had taken oral penicillin on several previous occasions without side-effects makes this possiblity less likely.

This case and others like it may represent a true bacterid. Treatment of bacterial infection may release certain bacterial antigens that stimulate an Arthus type reaction, and immune complexes deposited in the dermal vessels of the skin may then activate the complement system and result in vessel damage. The disorder is frequently self-limiting and usually resolves in 2–3 weeks.

REFERENCES

1 Baker H, Ryan TJ. Generalized pustular psoriasis A clinical and epidemiological study of 104 cases. *Br J Dermatol* 1968; 80: 771–93.

2 Tan RSH. Acute generalized pustular bacterid. *Br J Dermatol* 1974; **91:** 209–15.

3 Ishakawa H, Nameki H, Hattori H. *et al.* Akutes generisiertes pustuloses Bakterid. *Hautarzt* 1979; **30:** 144–8.

4 Burge SM, Ryan TJ. Acute palmoplantar pustolosis. *Br J Dermatol* 1984; **113:** 77–83.

5 Andrews GC, Birkman FW, Kelly RJ. Recalcitrant pustular eruptions of the palms and soles. *Arch Dermatol Syphilol* 1934; **29:** 548–63.

6 Andrews GC, Machacek GF. Pustular bacterids of the hands and feet. *Arch Dermatol Syphilol* 1935; **32:** 837–47.

7 Everall J. Intractable pustular eruptions of hands and feet. *Br J Dermatol* 1957; **69:** 269–72.

8 Ingram JT. Pustular psoriasis. *Arch Dermatol* 1958; **77:** 314–18.

9 Stevens DM, Ackerman AB. On the concept of bacterids (pustular bacterid, Andrews). *Amer J Dermatolopathol* 1984; **6:** 281–6.

10 Hellgren L, Mobacken H. Pustulosis palmaris et plantaris. *Acta Dermatovenereol* 1971; **51:** 284–8.

11 Baker H, Wilkinson DS. Persistant palmoplantar pustulosis. In: *Textbook of Dermatology* (Rook AJ, Wilkinson DS, Ebling FJ, eds), 3rd edn., Oxford: Blackwell Scientific Publications. 1979; 1365.

12 Soter NA, Mihm MC, Gigli I. *et al* Two distinct cellular patterns in cutaneous necrotizing angitiis. *J Invest Dermatol* 1976; **66:** 344–50.

13 Mackel SE, Tappeiner G, Brumfield H, Jordon RE. Circulating immune complexes in cutaneous vasculitis: detection with C1q and monoclonal rheumatoid factor. *J Clin Invest* 1979; **64:** 1652–60.

14 Alexander EL, Arnett FC, Provost TT, Stevens MB. Sjögren's syndrome: associtation on anti-Ro (SS-A) antibodies with vasculitis, haemotologic abnormalities, and serologic hyperreactivity. *Ann Intern Med* 1983; **98:** 155–9.

15 Alexander EL, Provost TT. Cutaneous manifestations of primary Sjögren's syndrome: a reflection of vasculitis and association with anti-Ro(SS-A) antibodies. *J Invest Dermatol* 1983; **80:** 386–91.

16 Kammer CM, Schur PH. Binding of circulating immune complexes to human peripheral blood lymphocytes: effect of complement. *Clin Immunol Immunopathol* 1978; **10:** 202–13.

17 Macmillin AL. Generalized pustular drug rash. *Dermatologica* 1973; **146:** 285–91.

18 Diaz LA, Provost TT, Tomasi TB. Pustular necrotizing angitis. *Arch Dermatol* 1973; **108:** 114–18.

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Intra-epidermal neutrophilic IgA dermatosis with colitis

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SUMMARY

We report the association of an intra-epidermal blistering disease of the skin with colitis. Both the skin and large bowel epithelium were infiltrated by neutrophils and eosinophils, and showed intra-epithelial deposition of IgA in an intercellular pattern. The colitis and the blisters responded well to treatment with dapsone.

CASE REPORT

A 73-year-old woman first came to the attention of the Skin Department in September 1983 when she was admitted to hospital following a stroke which had resulted in right-sided spastic hemiparesis and expressive dysphasia. At that time, she was diagnosed as having widespread endogenous eczema. This responded slowly to treatment with emollients, systemic anti-histamines and topical corticosteroid preparations, and she was not seen again by a dermatologist until May 1985. Her rash then consisted of a widespread scaly erythema mainly affecting the trunk and proximal parts of her legs, and contact sensitivity was suspected. The eruption settled, but at a further consultation in October 1985 grouped vesicles, some filled with pus, were noted (Fig. 1). Once again the rash settled without specific therapy. In November 1985, following a fall at home she was admitted to hospital for treatment of a broken hip, and again, multiple herpetiform pus-filled vesicles and larger flaccid bullae were seen. The skin cleared spontaneously before surgery, and her hip was repaired with a Thompson prosthesis.

During an otherwise uneventful recovery from surgery, she developed episodic bloody diarrhoea and a further widespread crop of blisters. These comprised both intact pus-filled bullae and eroded areas, all on a background of erythema. Lesions were again most marked on the trunk and proximal parts of the limbs (Fig. 2). At no stage had there been any oral or genital mucous membrane involvement.

Investigations

Haematological investigations showed haemoglobin levels varying between 11·3 and 13·0 g/dl,

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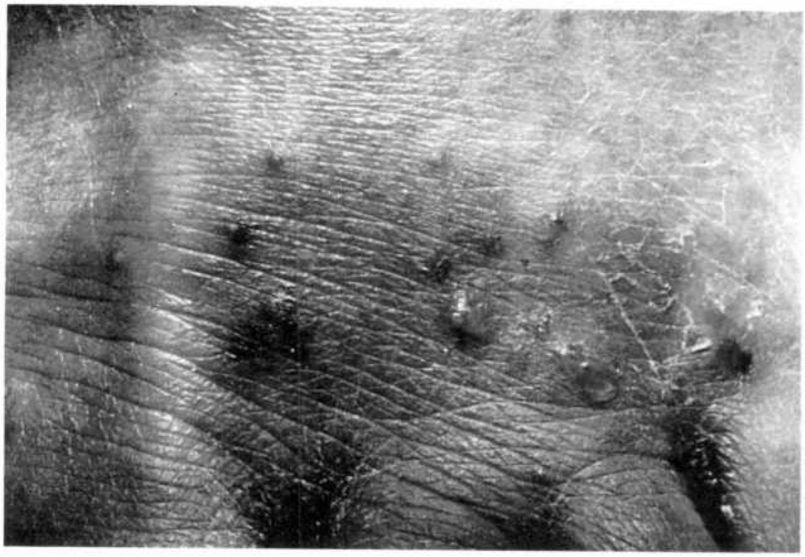

FIGURE 1. Grouped vesicles filled with pus seen on the dorsum of the forefoot.

with a total white blood cell count from 8·0–18·1 × 10^9 cells/l, always with a normal differential count. Erythrocyte sedimentation rate ranged from 49–106 mm/h. A full biochemical screen was normal on several occasions. Complement studies and tests for auto-antibodies were normal or negative, while serum immunoglobin levels included a raised IgA level of 485 mg/dl (normal range 60–350 mg/dl). No paraprotein was detected. Electron microscopy of blister fluid for virus particles was negative and swabs for virology and microbiology produced no growth.

Skin biopsy. Histology of lesional skin showed a bulla within the mid-epidermis containing some acantholytic cells and large numbers of neutrophil and eosinophil polymorphonuclear leukocytes. There was a moderate dermal eosinophilic infiltrate (Fig. 3).

Direct immunofluorescence of peri-lesional skin. This showed intra-epidermal supra-basal deposition of IgA (Fig. 4).

Rectal biopsy. A rectal biopsy showed superficial epithelial ulceration. The lamina propria contained lymphoid follicles with germinal centres and was heavily infiltrated with plasma cells and polymorphonuclear leukocytes. Well-developed crypt abscesses were seen.

Direct immunofluorescence of the rectal biopsy. This showed heavy uniform deposition of IgA in the mucosal epithelieum (Fig. 5).

Indirect immunofluorescence. Standard indirect immunofluorescence techniques were employed for the detection of circulating antibodies in the patient's serum (diluted 1:10) using guinea-pig lip, non light-exposed normal human skin and human colonic epithelium as substrates. There was no reaction with normal human skin, but circulating IgA was detected using fresh guinea-pig lip and normal human colonic epithelium. Indirect immunofluorescene using control serum was negative.

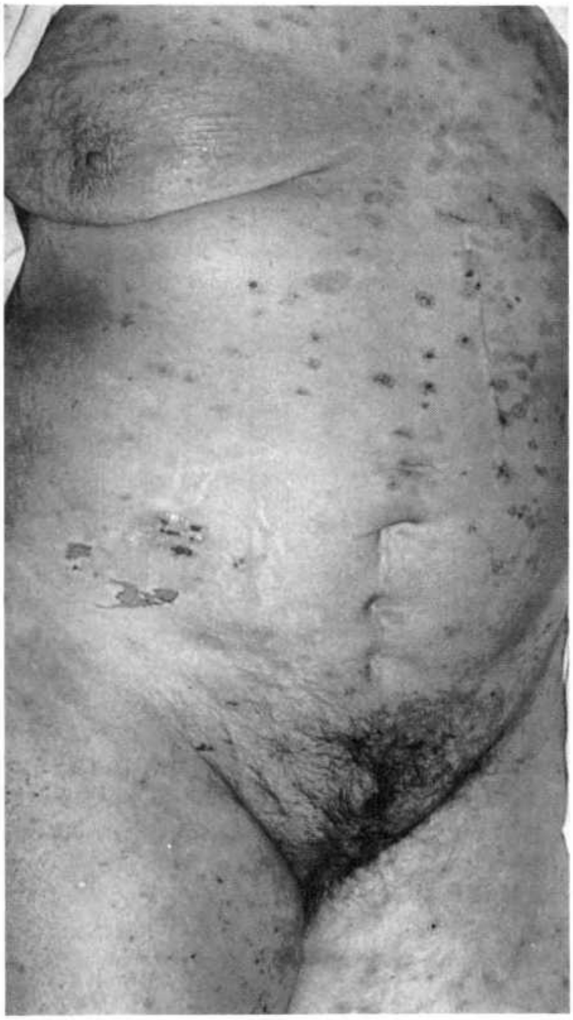

FIGURE 2. Vesicles and bullae on a background of erythema distibuted predominantly over the trunk and proximal parts of the limbs.

Treatment and progress

Following these investigations, the patient was treated with dapsone, 50 mg twice daily. Within 48 h there was marked resolution of gastrointestinal and cutaneous symptoms and signs. These symptoms recurred within 24 h of the drug being withdrawn, and resolved again when it was reinstituted. Over a follow-up period of 2 years, the minimum dosage of dapsone required to keep the patient symptom free was 50 mg twice weekly. There were no complications arising from the dapsone therapy.

DISCUSSION

The presence of intercellular IgA deposits in colonic epithelium and skin, the presence of a circulating IgA reactive with normal human colonic epithelium and guinea-pig lip and the rapid

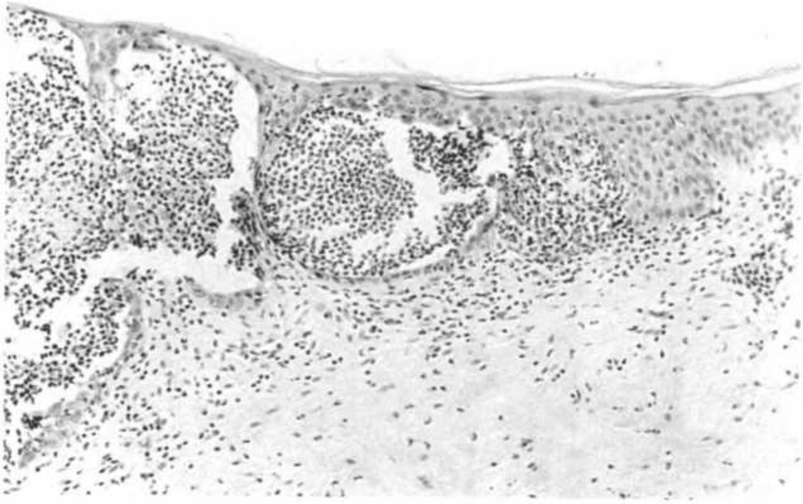

FIGURE 3. Histology of an intra-epidermal bulla. There is infiltration of the epidermis and underlying dermis by neutrophils and eosinophils. Some acantholysis can be seen. (Original × 200.)

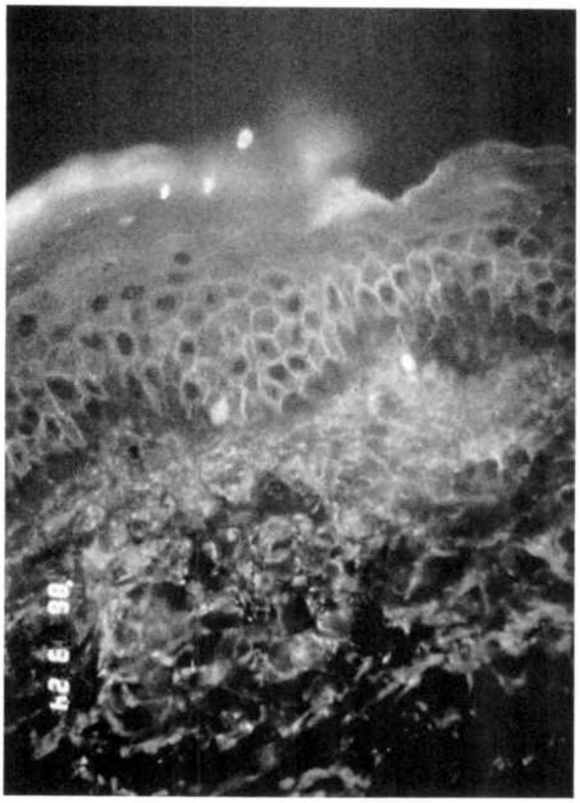

FIGURE 4. Direct immunofluorescence of peri-lesional skin showing intercellular deposition of IgA within the epidermis.

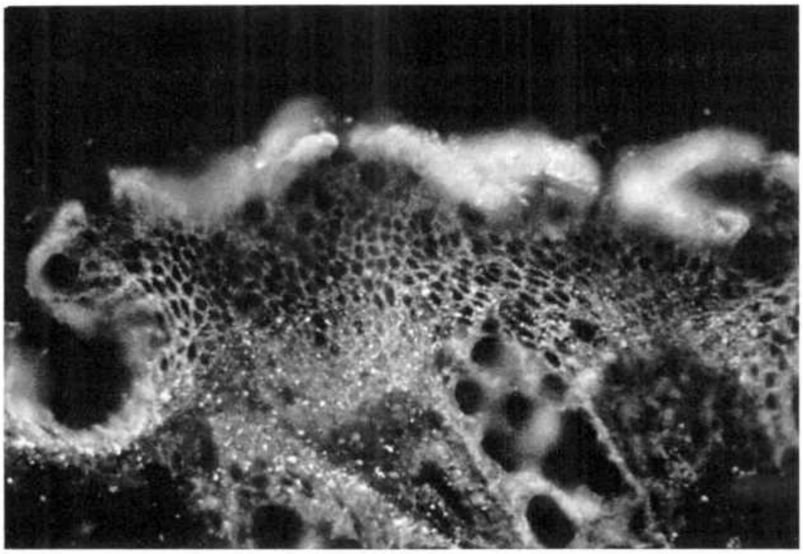

FIGURE 5. Direct immunofluorescence of rectal mucosa showing intercellular deposition of IgA within an ulcerated epithelium.

response to treatment with dapsone of both the gastrointestinal and the cutaneous symptoms, imply that the colitis and the cutaneous blisters were part of the same disease process. Our patient does not fit the description of any currently recognized bullous disease, but does resemble the two patients recently described as having 'intra-epidermal neutrophilic IgA dermatosis'.[1,2]

Huff *et al.*[1] reported a 70-year-old man with a predominantly truncal vesiculo-pustular rash and blisters located in the mid-epidermis. Neutrophils and eosinophils were seen both within the bulla and within the epidermis, and there was some acantholysis. The condition was highly responsive to dapsone. In the second case supra-basal acantholysis and intra-epidermal neutrophil abcesses in a 42-year-old man with a truncal pemphigus foliaceous-like rash were associated with pemphigus-like pattern of immunofluorescence and circulating IgA reactive with monkey oesophageal epithelium. The authors considered this to be the second example of 'intra-epidermal neutrophilic IgA dermatosis'.[2]

The principal differential diagnosis of intra-epidermal neutrophilic IgA dermatosis is sub-corneal pustular dermatosis (SPD), the hallmark of which is the strictly sub-corneal pustule filled with polymorphonuclear leukocytes and only an occasional eosinophil.[3] While the mid-epidermal location of the blisters in our patient and the predominance of eosinophils in the cellular infiltrate serve to distinguish the condition from SPD, there are also distinguishing clinical features. The distribution of blisters in our patient was mainly truncal, whereas SPD characteristically affects the flexures. In general, SPD responds slowly to treatment with sulphones, whereas our patient responded dramatically.

There is a spectrum of blistering diseases associated with neutrophilic infiltrates and the intra-epidermal deposition of IgA. In these, IgA deposition may occur in one of three patterns. Firstly, linear sub-corneal deposition has been noted in two patients. Wallach *et al.*[4] reported a 92-year-old woman with a pustulobullous eruption considered histologically typical of sub-corneal pustular dermatosis. An IgA kappa light chain monoclonal gammopathy was present.

The same immunofluorescence pattern was seen in a 26-year-old woman with a vesiculopustular eruption associated with fever and polyarthritis.[5] In the second of these patients, both the cutaneous and articular symptoms responded rapidly to treatment with dapsone.

Secondly, IgA was noted on polymorphs in the sub-corneal pustules of a patient with SPD by Sneddon and Wilkinson.[3] They were unable to find IgA in their other patients with the condition, and concluded that it was likely to be artefactual.

Finally, a pemphigus-like pattern of IgA deposition has been found in five cases reported to date. Three of these have been considered to be examples of SPD, with characteristic sub-corneal pustules.[6,7,8] Two of these three patients also had an IgA gammopathy. The other two cases were reported as intra-epidermal neutrophilic IgA dermatosis.

The relationship between IgA and diseases of the skin and gastro-intestinal tract is intriguing. The combination of intra-epithelial IgA deposits in both the large bowel and the skin with infiltration of the epithelium by neutrophils and eosinophils is, to our knowledge, unique to our patient. In dermatitis herpetiformis complicating coeliac disease, IgA is deposited in the very superficial dermis, and blisters are sub-epidermal.[9] An IgA gammopathy has been described in association with inflammatory bowel disease complicated by pyoderma gangrenosum.[10] Pyoderma gangrenosum has, in turn, been asociated with sub-corneal pustular dermatosis and a monoclonal IgA dermatosis.[11-13]

The presence of polymorphonuclear leukocytes (PMNL) in the inflammatory infiltrate of these conditions is characteristic, although the simultaneous occurrence of both IgA and PMNL in the infiltrate is of unknown significance. Neutrophils and eosinophils are found in both sub-epidermal and intra-epidermal blistering diseases, regardless of whether IgA is present.

The mechanism of the therapeutic action of dapsone in these diseases is unknown, and may be due to its inhibitory effect on PMNL cytotoxicity.[14] Although dapsone is a potent inhibitor of potassium iodide-induced pustule formation *in vivo*,[15] *in vitro* studies have failed to show a direct effect of the drug on PMNL chemotaxis.[16] Recent studies indicate that dapsone may act as a quencher of oxygen intermediates as well as an inhibitor of the myeloperoxidase-H_2O_2-halide system,[17] although a role for oxygen derived free radicals has not so far been claimed in the bistering diseases characterized by PMNL infiltrates. Furthermore, dapsone has been shown to inhibit the production of pro-inflammatory eicosanoids from arachidonic acid in the rat mast cell.[18]

To propose that the IgA is central to the pathogenesis of the blisters in the condition reported here would be premature, and further advances in the understanding of the role of IgA await characterization of the antigen. This case extends the clinical spectrum of IgA dermatoses and adds another link to the chain of association between inflammatory disease of the skin and the gastrointestinal tract.

REFERENCES

1 Huff JC, Golitz Le, Kunke KS. Intraepidermal neutrophilic IgA dermatosis. *N Engl J Med* 1985; **313**: 1643–5.

2 Piette WW, Burken RR, Ray TI. Intraepidermal neutrophilic IgA dermatosis: presence of circulating pemphigus-like antibody specific for monkey epithelium (Abstact). *J Invest Dermatol* 1987; **88**: 512

3 Sneddon IB, Wilkinson DS. Subcorneal pustular dermatosis. *Br J Dermatol* 1979; **100**: 61–68

4 Wallach D. Cottenot P, Pelbois G *et al* Subcorneal pustular dermatosis and moncolonal IgA. *Br J Dermatol* 1982; **107**: 229–34.

5 Wallach D, Janssen FM, Vignon-Pennamen M-D *et al.* Atypical neutrophilic dermatosis with sub-corneal IgA deposits. *Arch Dermatol* 1987; **123**: 790–5.

6 Wallach D, Foldes C, Cottenot F. Pustulose sous-cornee, acantholyse superficielle et IgA monoclonale. *Ann Dermatol Venereol* 1982, **109:** 959–63.

7 Tagami H, Iwatsuki K, Iwase Y *et al.* Subcorneal pustular dermatosis with vesiculo-bullous eruption: demonstration of subcorneal IgA deposits and a leukocyte chemotactic factor. *Br J Dermatol* 1983; **109:** 581–7.

8 Burrows D, Bingham EA. Subcorneal pustular dermatosis and IgA gammopathy. *Br J Dermatol* 1984; **111** (Suppl. 26): 91–3.

9 Katz SI, Strober W. The pathogenesis of dermatitis herpetiformis. *J Invest Dermatol* 1978; **70:** 63–75.

10 Powell FC, Schroeter AL, SU WPD Pyoderma gangrenosum and monoclonal gammopathy. *Arch Dermatol* 1983, **119:** 468–72.

11 Wolfe K. Subkorneale pustulose Dermatose (Sneddon-Wilkinson): Pyoderma Gangrenosum mit IgA paraproteinamie. *Dermatol Monatsschr* 1971; **157:** 842.

12 Marsden JR, Millard LG. Pyoderma gangrenosum, subcorneal pustular dermatosis and IgA paraproteinaemia. *Br J Dermatol* 1986; **114:** 125–9.

13 Venning VA, Ryan TJ. Subcorneal pustular dermatosis followed by pyoderma gangrenosum. *Br J Dermatol* 1986; **115:** 117–18.

14 Stendahl O, Molin L, Dahlgren C. The inhibition of polymorphonuclear leucocyte cytotoxicity by dapsone. *J Clin Invest* 1979; **62:** 214–20.

15 Plewig G, Schopf E. Anti-inflammatory effects of antimicrobial agents: an *in vivo* study. *J Invest Dermatol* 1975; **65:** 532–6.

16 Esterly NB, Furey NL, Flannagan BS. The effect of antimicrobial agents on leukocyte chemotaxis. *J Invest Dermatol* 1978; **70:** 51–5.

17 Niwa Y, Sakane T, Miyachi, Y. Dissociation of the inhibitory effect of dapsone on the generation of oxygen intermediates—in comparison with that of colchicine and various scavengers. *Biochem Pharmacol* 1984; **33:** 2355–9.

18 Ruzicka T, Wasserman SI, Soter NA *et al.* Inhibition of rat mast cell arachidonic acid cyclo-oxygenase by dapsone. *J Allergy Clin Immunol* 1983; **72:** 365–70.

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Lymphocytic lymphoma and acquired C_1 esterase inhibitor deficiency

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SUMMARY

We report a 59-year-old woman who had progressive symptoms of angioedema for 3 years, culminating in an attack of laryngeal stridor. C_1 esterase inhibitor deficiency was confirmed and treatment with tranexamic acid produced considerable clinical benefit and improvement in complement levels. Two years later she developed clinical evidence of lymphocytic lymphoma. Splenectomy resulted in rapid correction of complement abnormalites. The patient received 6 months of chemotherapy after surgery and remained asymptomatic with normal complement levels 4 years later.

Hereditary angioedema is caused by a quantitative or qualitative defect of complement C_1 esterase inhibitor (C_1INH). It is characterized by recurrent episodes of subcutaneous and mucosal oedema which is brawny and non-pruritic, involving the extremities, face, abdominal viscera and airway.

Several cases of acquired C_1INH deficiency have now been reported in association with lymphoproliferative diseases.[2–5]

We describe here a case of acquired C_1INH deficiency in which the symptoms preceded the clinical diagnosis of lymphocytic lymphoma by 5 years.

CASE REPORT

A 59-year-old woman was admitted as an emergency in October 1981 with facial swelling and stridor. She had suffered from intermittent episodes of limb or facial swelling, often with diarrhoea, for 3 years, the symptoms becoming increasingly severe. Physical examination revealed no abnormality apart from facial oedema and laryngeal stridor. The blood count on admission was normal (haemoglobin 12·8 g/dl, white cell count $6{\cdot}0 \times 10^9$/l, with a normal differential count). Investigations confirmed C_1INH deficiency (C_1INH 0·08 g/l; normal range

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TABLE 1. Levels of C_1INH, C_4 and C_3 at diagnosis, after 2 years tranexamic acid therapy* and following splenectomy

	C_1INH (g/l)	C_4 (g/l)	C_3 (g/l)
October 1981	0·08	0·01	0·88
December 1983			
Pre-operative*	0·23	0·13	0·78
day 0 (6 h post-operative)	0·28	0·20	0·89
day 2	0·34	0·24	0·92
day 3	0·37	0·28	1·05
day 5	0·50	0·32	1·37
day 6	0·53	0·32	1·36
day 7	0·59	0·45	1·25
day 21	0·60	0·32	1·41
day 54	0·70	0·47	1·06
January 1985	0·25	0·49	0·90
January 1986	0·30	0·34	1·22
September 1987	0·31	0·42	1·50
Normal range	(0·18–0·26)	(0·14–0·54)	(0·75–1·75)

0·18–0·26 g/l) with depletion of early complement components (C_4, 0·01 g/l; normal range 0·14–0·54 g/l) and with a normal C_3 level (0·88 g/l; normal range 0·75–1·75 g/l).

Because of her serious and frequent attacks, she was prescribed continuous tranexamic acid 500 mg tds, and apart from two minor episodes of facial swelling she remained well.

Two years later, she was noted to be pale and on examination had marked splenomegaly, but no palpable lymphadenopathy. A blood count showed haemoglobin 9·6 g/dl, white cell count $2·4 \times 10^9$/l with a normal differential count, and platelets 96×10^9/l. Immunoglobulin levels were low: IgG, 4·7 g/l (normal range 7·0–19·0 g/l), IgA, 0·8 g/l (normal range 1·0–4·5 g/l), IgM, 0·18 g/l (normal range 0·5–2·0 g/l) and no paraprotein band detected. Bone marrow biopsy showed multiple foci of well-differentiated lymphocytic lymphoma.

At laparotomy a spleen weighing 5·4 kg was removed. There was no evidence of abdominal lymphadenopathy at operation. Serial C_1INH, C_4 and C_3 levels are shown in Table 1. The patient was subsequently treated with intermittent chemotherapy (Vincristine, chlorambucil and prednisolone) for 6 months, and tranexamic acid was stopped 1 year after surgery. The patient remained well and clinically free of lymphoma, had no further attacks of angioedema and had normal complement and C_1INH levels 4 years after splenectomy.

DISCUSSION

The mechanism of complement depletion in acquired angioedema is uncertain. Caldwell *et al.*[2] found low levels of complement C_1 in two cases of acquired disease associated with lymphoma, whereas in the hereditary form C_1 levels are normal. It has been proposed that the low levels of C_1 and other early complement components C_4 and C_2 reflect a primary excessive activation of the complement cascade and that low C_1INH levels are due to consumption, secondary to this activation. Many of the early cases of lymphoma associated with angioedema had a demonstrable IgM paraprotein[2,4,6] and serum mixing experiments showed complement activation in normal serum. Schreiber *et al.*[5] demonstrated complement C_1 depletion in normal serum using a cell suspension from lymphoma tissue of a patient with acquired angioedema and lymphoproliferative disorder.

Recently, cases of acquired C_1INH deficiency have been described where low activity is not the result of excessive consumption, but of impaired binding of C_1INH to activated C_1 caused by acquired IgG antibodies to C_1INH.[7,8] These cases, however, have not been associated with lymphoma or any other accompanying disease. C_1INH levels measured immunologically are only slightly reduced (60–70% of normal), but activity is severely impaired in functional assays.

Splenectomy in lymphoma is accompanied by cessation of attacks of angioedema and rapid reversal of complement abnormalities[9,10] although the effects may be only transient.[10] Gelfand *et al.*[11] showed similar beneficial effects of splenectomy in a patient with autoimmune haemolytic anaemia in whom there was no demonstrable lymphoma or other haematological malignancy.

It is uncertain, therefore, whether the effect of splenectomy is simply due to removal of tumour mass or whether there is some other modifying spleen factor as yet unidentified. Acquired C_1INH deficiency has also been described in a patient with myelofibrosis and splenomegaly.[12]

Danazol, a semi-synthetic androgen, has been used successfully in the treatment of hereditary angioedema.[13] Androgens may act by increasing production of C_1INH. C_4 levels in treated patients often return to normal. In acquired cases, danazol had reduced the frequency and intensity of attacks, but C_1, C_4 and C_2 levels may remain low.[14,15] Epsilon aminocaproic acid (EACA) has also been used in hereditatry angioedema.[16] This agent (and tranexamic acid) inhibits activated C_1.[17] Since the defect in the acquired form is probably direct activation of C_1 rather than defective production of C_1INH, the use of EACA or tranexamic acid seems more appropriate than danazol, but there are risks of thromboembolic complications.

In the patient we report here, symptoms of angioedema preceded the diagnosis of her lymphoma by 5 years. We did not measure her complement C_1 level, but there was no family history, and other family members tested were normal. Neither did we attempt to detect autoantibodies to C_1INH, but the very low level of inhibitor at diagnosis was in keeping with excessive consumption rather that defective binding.[8] Treatment with tranexamic acid prevented all, but two minor attacks, and while C_4 levels remained slightly low, C_1INH levels returned to normal.

Splenectomy resulted in the removal of the bulk of the tumour tissue. Within 6 h of operation the C_4 level was normal. C_1INH and C_4 levels continued to rise to above normal levels over the 7 post-operative days, and higher than normal levels were still detected 2 months after splenectomy. C_4 and C_1INH levels were normal 2, 3 and 4 years later.

Patients who develop symptoms of anigoedema as adults may have an underlying lymphoma or other malignancy, including rectal carcinoma[15] and perhaps small cell carcinoma of the lung[18] or myelofibrosis.[12] Symptoms may precede detectable manifestations of the associated malignancy,[19] as occurred in our case. Treatment with cyclokapron or EACA, which inhibit early complement activation, may, despite thrombotic risks, be more appropriate than danazol in correcting complement levels and preventing symptoms of angioedema.

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REFERENCES

1 Donaldson VH, Evans RR. A biochemical abnormality in hereditary angioneurotic edema: absence of serum inhibitor of C_1 esterase. *Am J Med* 1963; **35:** 37–44.

2 Caldwell JR, Ruddy S, Schur PH, Austen KF. Acquired C_1 inhibitor deficiency in lymphosarcoma. *Clin Immunol Immunopathol* 1972; **1:** 39–52.

3 Hauptmann G, Lang JM, North ML *et al.* Acquired C_1 inhibitor deficiencies in lymphoproliferative disease with serum immunoglobulin abnormalities. *Blut* 1976; **32:** 195.

4 Day NK, Winfield JF, Winchester, RJ *et al.* Evidence of immune complexes involving antilymphocyte antibodies associated with hypocomplementemia in chronic lymphatic leukaemia (C.L.L.). *Clin Res* 1975; **23:** 410

5 Schreiber, AD, Zweiman B, Atkins P. *et al.* Acquired angioedema with lymphoproliferative disorder; association of C_1 inhibitor deficiency with cellular abnormality. *Blood* 1976; **48:** 567–80.

6 Rosenfeld SI, Staples PJ, Leddy JP. Angioedema and hypocomplementemia; unusual features of lymphoma. *J Allergy Clin Immunol* 1975; **55:** 104.

7 Jackson J, Sim RB, Whelan A, Feighery C. An IgG autoantibody which inactivates C_1-inhibitor. *Nature* 1986; **323:** 722–4.

8 Alsenz J, Bork K., Loos M. Autoantibody-mediated acquired deficiency of C_1 inhibitor. *N Engl J Med* 1987; **316** (22): 1360–6.

9 Hauptmann G, Peititjean F, Lang, JM, Oberling F. Acquired C_1 inhibitor deficiency in a case of lymphosarcoma of the spleen. Reversal of complement abnormalities after splenectomy. *Clin Exp Immunol* 1979; **37:** 523–31.

10 Whaley K, Balogh Z Sjögren's syndrome: a genetically determined exocrinopathy associated with lymhoreticular neoplasia and abnormalities of the serum complement system. In: *Advanced Medicine* 16 (Bellingham AJ, ed) Pitman Medical Publications Ltd., 1980 287–95.

11 Gelfand JA, Boss GR, Conley CL *et al.* Acquired C_1 esterase inhibitor deficiency and angioedema: a review. *Medicine (Baltimore)* 1979; **58:** 321–8.

12 Nilsen A, Matre R. Acquired angioedema and hypocomplementemia in a patient with myelofibrosis. *Acta Med Scand* 1980; **207:** 123–5.

13 Gelfand JA, Sherins RJ, Alling DW, Frank MM. Treatment of hereditary angioedema with danazol. *N Eng J Med* 1976; **295:** 1444–8.

14 Hauptmann G, Mayer S, Lang JM *et al.* Treatment of acquired C_1 inhibitor deficiencey with danazol. *Ann Intern Med* 1977; **87:** 577–8.

15 Cohen DH, Koethe SM, Kozin F *et al.* Acquired angioedema associated with rectal carcinoma and its response to danazol therapy. *J All Clin Immunol* 1978; **62:** 217–21.

16 Frank MM, Sergent JS, Kane MA, Alling DW. Epsilon aminocaproic acid therapy of hereditary angioneurotic oedema. A double-blind study. *N Engl J Med* 1972; **286:** 808–12.

17 Soter NA, Austen KF, Gigli I. Inhibition by E-aminocaproic acid of the activation of the first component of the complement system. *J Immunol* 1975; **114:** 928–32.

18 Ross TF, Coleman DL, Naughton JL. Angioedema and small-cell carcinoma of the lung. *Thorax* 1982; **37:** 950–1.

19 Chevant-Breton J, Mazeas D, Bagot M *et al.* Acquired angioneurotic oedema caused by acquired deficiency of C_1 esterase inhibitor disclosing lymphoproliferative syndrome. A propos of a case, review of the literature. *Ann Dermatol Venereol* 1982; **109:** 1049–56 (English abstract).

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Society Proceedings

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HOST: PROFESSOR DR R.HAPPLE

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CASE REPORTS

The hyper IgE syndrome and generalized molluscum contagiosum

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History. A 17-year-old girl was seen who had had atopic dermatitis and relapsing infections of the respiratory tract since early childhood. At the age of 6 she had had mollusca contagiosa for the first time. During the last 5 years she had been given prednisone orally at regular intervals for treatment of her severe atopic dermatitis.

Examination. The girl presented with a skeletal age of 12 years. The entire skin was covered with 5 to 10 mm lesions of molluscum contagiosum (Fig. 1). Further inspection of the skin revealed a mild atopic dermatitis and some abscesses localized to the nates.

Investigations. IgE was markedly increased (48,000 IU/ml) and a differential white cell count showed eosinophilia (20%). Humoral and cellular immunity was abnormal. Endocrinological investigations revealed adrenal-axis suppression.

Comment. The hyper IgE syndrome seems responsible for this extensive manifestation of molluscum contagiosum. It is conceivable, however, that the prolonged intake of prednisone is also of relevance in this respect.

Keratosis palmoplantaris transgrediens et progrediens Greither

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History. A 44-year-old woman presented with progressive palmoplantar keratosis. The hyperkeratotic areas became tender on wetting. The patient's son had similar abnormalities.

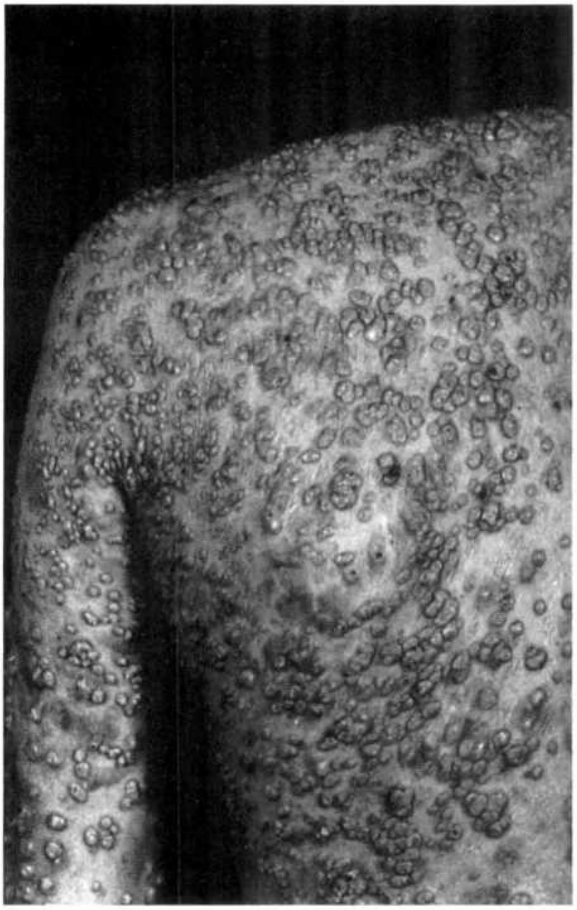

FIGURE 1. Generalized mollusca contagiosa.

Examination. The volar and interdigital aspects of the fingers showed a sharply demarcated verrucous hyperkeratosis, which encroached on the dorsa (Fig. 2). The palms and soles showed moderate palmoplantar keratosis.

Histology and electron microscopy. The hyperkeratosis was orthokeratotic with a markedly thickened stratum granulosum.

Comment. The progressive hyperkeratosis, spreading to the dorsal surfaces of the fingers, and being less prominent on the palms and soles, and the positive family history are compatible with the diagnosis of keratosis palmoplantaris transgrediens et progrediens Greither.

REFERENCES

1 Greither A. Keratosis extremitatum hereditaria progrediens mit dominantem Erbgang. *Hautarzt* 1952; **3:** 198–203.
2 Grin E, Salamon T, Miliveci M. Keratosis palmo-plantaris mit dominantem Erbgang. *Arch Klin Exp Dermatol* 1962; **214:** 378–93.

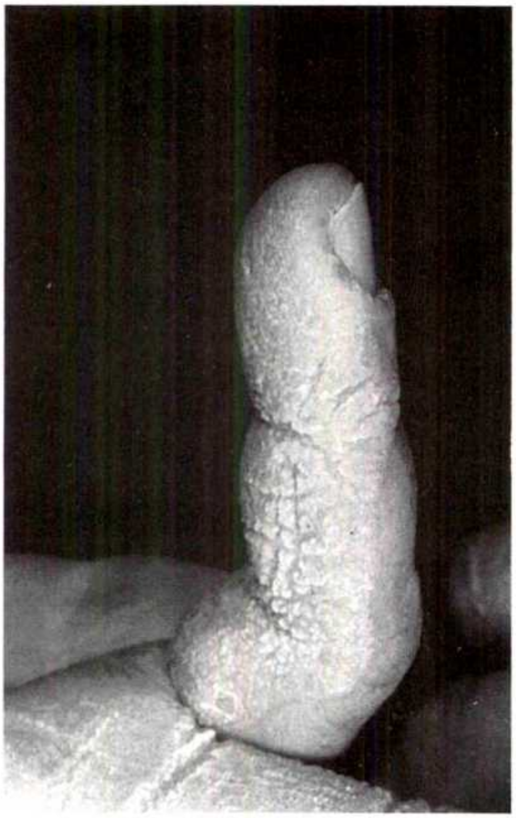

FIGURE 2. Palmoplantar keratoderma of the Greither type.

Bullous acrokeratotic poikiloderma of the Kindler-Weary type

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History. A 26-year-old woman presented with a bullous eruption. Shortly after birth multiple bullae had appeared. During the last 6 months bullae were restricted to the feet. During childhood she had developed a dry skin with areas of hyperpigmentation in the axillae.

Examination. In the axillary regions reticular areas of hyperpigmentation were seen. Small areas of hyper- and hypopigmentation were present on the arms and legs. Yellow-brown keratotic papules were observed on the hands.

Histology. A sub-epidermal bulla and a band-like mononuclear infiltrate was seen. Immunohistological examination revealed, apart from deposits of fibrinogen within and around the bulla, only minimal amounts of complement in the vessel walls.

Comment. The co-existence of bullae, keratotic papules on the acral areas, and poikiloderma has been described by Kindler[1] and Weary *et al.*[2] A review of the literature is provided by Larrègue *et al.*[3]

REFERENCES

1 Kindler T. Congenital poikiloderma with traumatic bulla formation and progressive cutaneous atrophy. *Br J Dermatol* 1954; **66:** 104–11.

2 Weary PE, Manley WF, Graham GF. Hereditary acrokeratotic poikiloderma. *Arch Dermatol* 1971; **103:** 409–22.

3 Larrègue M, Prigent F, Lorette G *et al.* Acrokératose poikilodermique bulleuse et héréditaire de Weary-Kindler. *Ann Dermatol Venereol (Paris)* 1981; **108:** 69–76.

X-linked dominant chondrodysplasia punctata (10-year follow-up)

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History. A 25-year-old woman had had since birth a shortening of the right leg with contracture of the knee-joint and dislocation of the patella. X-ray examination had revealed stippled calcifications of the epiphyses. At birth the skin had shown irregular areas of erythema and scaling which had disappeared after a few weeks. The family history was negative.

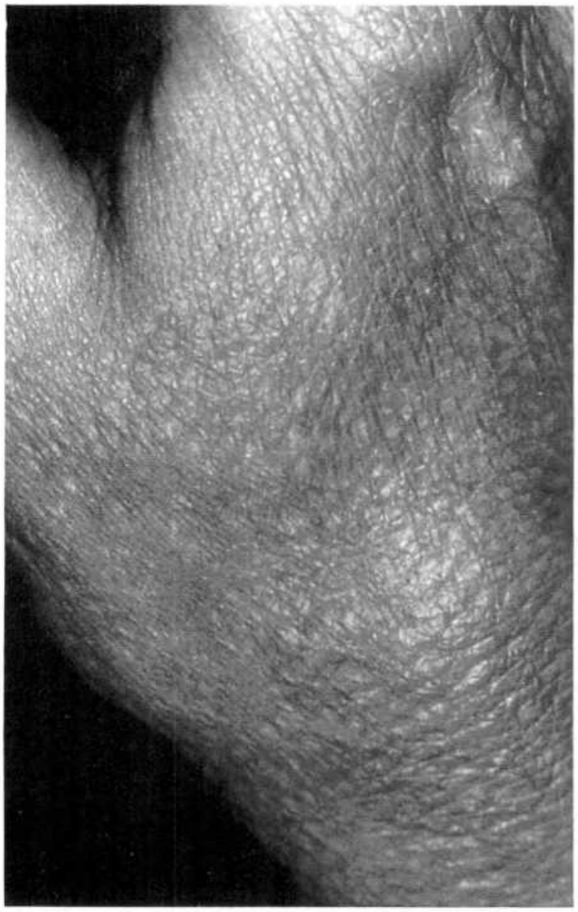

FIGURE 3. X-linked dominant chondrodysplasia punctata.

Examination. Scoliosis and shortening of the right leg caused a marked reduction of the body length. Ichthyotic scaling was seen, especially on areas of follicular atrophoderma (Fig. 3). The distribution of these changes was asymmetrical. Scalp hair was coarse and twisted and two areas of alopecia were seen. Examination of the eyes revealed bilateral cataracts. On the left side the opacities resembled ice crystals and on the right side the distribution was sectorial.

Comment. X-linked dominant chondrodysplasia punctata is characterized by skeletal, ocular and cutaneous anomalies with an asymmetrical distribution.[1] This was the first case in which the X-linkage was recognized because of the linear and patchy distribution of the skin lesions, which suggested functional X-chromosome mosaicism.[2]

REFERENCES

1 Happle R. X-linked dominant chondrodysplasia punctata. Review of literature and report of a case. *Hum Genet* 1979; **53:** 65–73.

2 Happle R, Matthias HH, Macher E. Sex-linked chondrodysplasia punctata? *Clin Genet* 1977; **11:** 73–6

Congenital hereditary hypotrichosis of the Marie Unna type

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History. A 29-year-old man had a tonsure-like bald area on the occiput. Eyebrows and eyelashes had been partially lacking since childhood. His sister and his father presented with similar changes. His paternal grandmother and great-grandfather had had similar abnormalities.

Examination. The scalp showed a more than palm-sized area of alopecia in the parieto-occipital region, in which no hair follicles were observed. The remaining hairs were coarse. Some hair shafts were bent irregularly. Eyebrows, eyelashes and body hair were sparse.

Microscopy. Hair shafts of this patient, his sister and his father showed alternating thick and thin stretches and were irregularly rotated. If we pulled a hairshaft gently, it developed a typical kink, as can be produced by stretching an iron wire.

Comment. The pattern of transmission, the clinical picture and the microscopic findings are typical of congenital hereditary hypotrichosis of the Marie Unna type.[1,2]

REFERENCES

1 Unna M. Über Hypotrichosis congenita hereditaria. *Dermatol Wochenschr* 1925; **32:** 1167–78.

2 Ludwig E. Hypotrichosis congenita hereditaria Typ Marie Unna. *Arch Dermatol Syph* 1953; **196:** 261–78.

Proteus syndrome

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History and examination. A 17-year-old man presented with an extensive naevus flammeus on

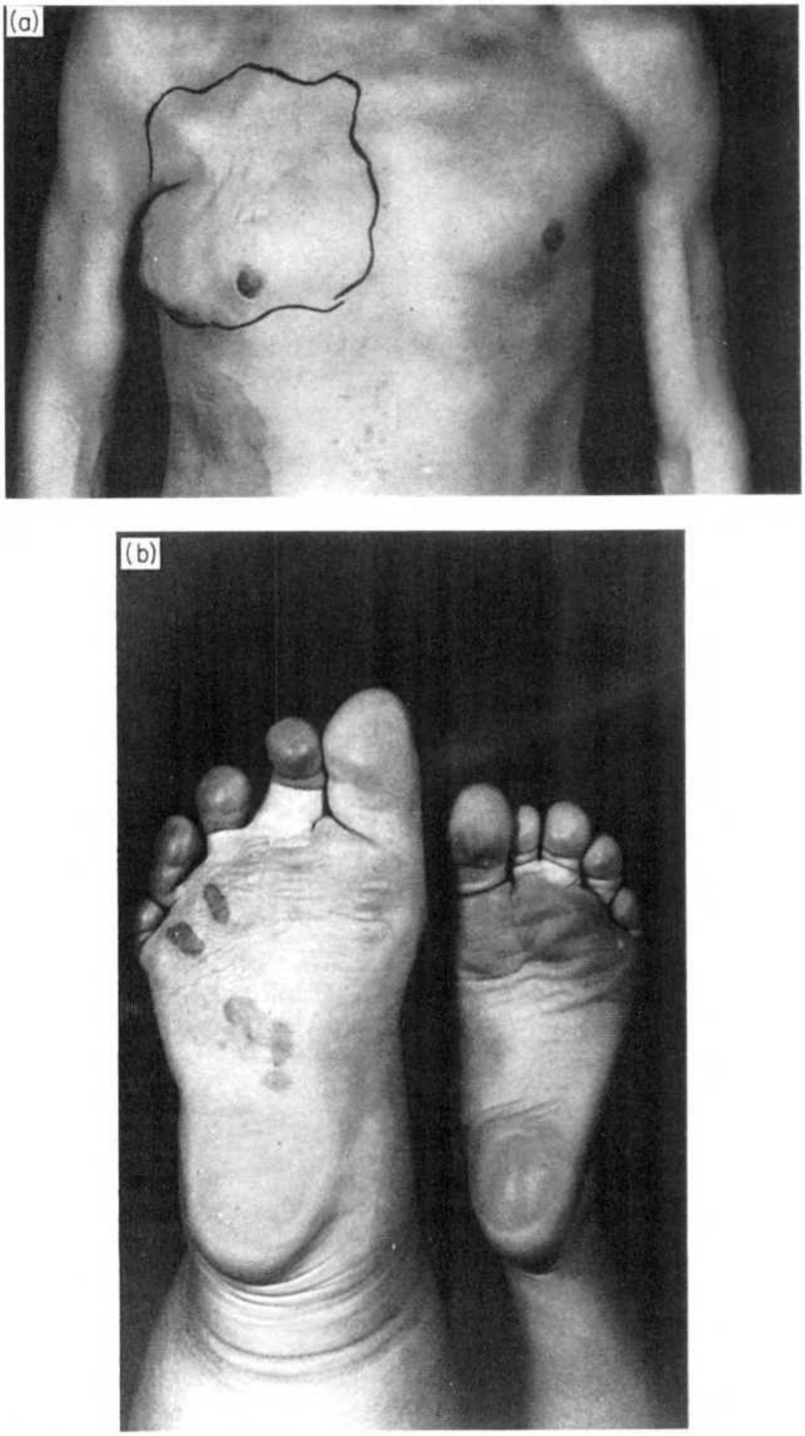

FIGURE 4. Proteus syndrome: (a) Pectoral hypertrophy and naevus flammeus; (b) cavernous lymphangioma causing hypertrophy of right leg.

his right side. A subcutaneous soft tissue tumour was palpable on the right side of his chest (Fig. 4a). During the 1st years of life the right leg became hypertrophic (Fig. 4b). In 1979 a successful epiphysiodesis was carried out. A pronounced vena lateralis marginalis perstans was seen on his right leg, and marked macrodactylia of his right foot and asymmetry of the fingers was observed.

Histology. The tumour on the chest proved to be a cavernous angioma with empty lumina. Therefore, a diagnosis of cavernous lymphangioma was made.

Comment. The osteohypertrophy of the right leg, the naevus flammeus and the varicosis would be compatible with the diagnosis of Klippel-Trenaunay syndrome. The symmetrical macrodactylia and the lymphangioma of the chest, however, do not support this diagnosis. All the abnormalities seen in this patient are compatible with the diagnosis of Proteus syndrome.[1-4]

REFERENCES

1 Wiedemann HR, Burgio GR, Aldenhoff P *et al.* The Proteus syndrome. Partial giantism of the hands and/or feet, nevi, hemihypertrophy, subcutaneous tumors, macrocephaly or other skull anomalies and possible accelerated growth and visceral affections. *Eur J Pediatr* 1983; **140:** 5–12.

2 Viljoen DL, Nelson MM, de Jong G, Beighton P. Proteus syndrome in Southern Africa: Natural history and clinical manifestations in six individuals. *Am J Med Genet* 1987; **27:** 87–97.

3 Clark RD, Donnai D, Rogers J *et al.* Proteus syndrome: an expanded phenotype. *Am J Med Genet* 1987; **27:** 99–117.

4 Malamitsi-Puchner A, Kitsiou S, Bartsocas CS. Brief clinical report: Severe Proteus syndrome in an 18-month-old boy. *Am J Med Genet* 1987; **27:** 119–25.

Schöpf syndrome

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History. A 75-year-old man was seen with tumours on the hands. At the age of 18 years, he had been fitted with a denture because of hypoplastic teeth. As a student he developed alopecia of the scalp, and later he developed palmoplantar hyperkeratosis and cysts on the eyelids. Eight years ago, the fourth finger of his left hand was amputated because of a squamous cell carcinoma on the terminal phalanx.

Examination. There was marked hypotrichosis of the scalp with follicular hyperkeratosis, palmoplantar hyperkeratosis with erythema, scaling and fissuring, a solid tumour in the first interdigital space of the right hand, a palmar hyperkeratotic papule, and multiple cysts on the eyelid margins.

Histology. The interdigital tumour and the palmar papule were diagnosed as a squamous cell carcinoma and a premalignant keratosis with pronounced atypia, respectively. The cysts on the eyelids were found to be hidrocystomas.

Comment. In 1971 Schöpf *et al.*[1] reported two sisters affected by a new syndrome, consisting of eyelid cysts, palmoplantar hyperkeratosis, hypodontia and hypotrichosis.[1] In 1984 Burket *et al.*[2] described a third patient. To the best of our knowledge, this is the third report on this syndrome. It would appear that this syndrome can be added to the genodermatoses associated with cancer-proneness.

REFERENCES

1 Schöpf E, Schulz HJ, Passarge E. Syndrome of cystic eyelids, palmoplantar keratosis, hypodontia and hypotrichosis as a possible autosomal recessive trait. *Birth Defects* 1971; **8:** 219–21.

2 Burket JM, Burket BJ, Burket DA. Eyelid cysts, hypodontia, and hypotrichosis. *J Am Acad Dermatol* 1984; **10:** 922–5.

Congenital cutaneous ossification

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History. A 1-year-old girl presented with indolent 'bony' structures which had been observed in the skin shortly after birth.

Examination. Irregularly shaped bony structures were present on her left shoulder and arm and also on the lower part of her right leg (Fig. 5). In some places the overlying skin showed some redness and atrophy.

Histology. Several homogeneous calcified areas with a sharp edge were seen intradermally. Within these areas there were many cells with a perinuclear halo. No Haversian channels were observed.

Comment. Most authors are convinced that cutaneous ossification can be either a primary or a secondary process.[1,2] Others deny the existence of the primary form. Although we did not find any pre-existent skin lesion or a systemic disease, such as Albright's hereditary osteodystrophy, in our patient, we cannot prove that we are dealing with primary ossification. We, therefore, prefer to classify this case as 'congenital cutaneous ossification'.

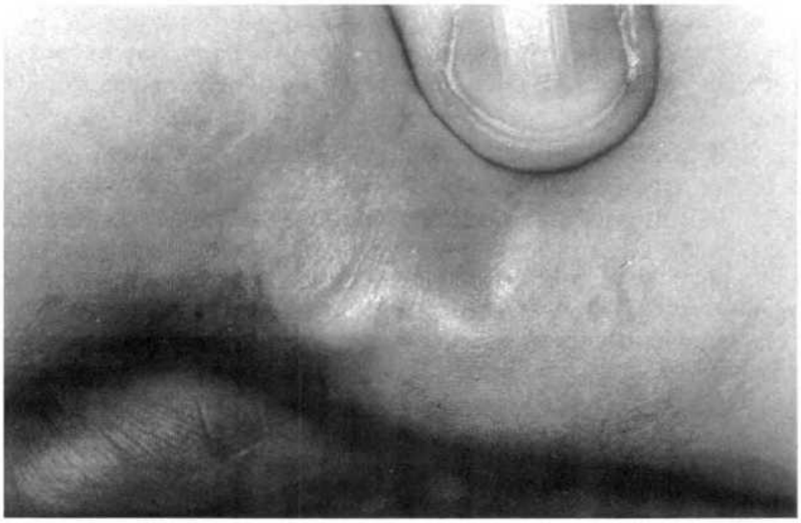

FIGURE 5. Congenital cutaneous ossification.

REFERENCES

1 Nakamura S, Imai T, Nakayama K, Onda S. Primary osteoma cutis. *J Dermatol (Tokyo)* 1987; **14:** 85–8.
2 Roth SI, Stowell RE, Helwig EB. Cutaneous ossification. Report of 120 cases and review of the literature. *Arch Pathol* 1963; **76:** 44–53.

SUMMARIES OF PAPERS

What is new in psoriasis research?

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Psoriasis research focusses on control of inflammation and proliferation. Arachidonic acid and its derivatives play an important role in this respect. In the lesion as well as in the clinically uninvolved skin, production of these mediators is increased.

In the clinically uninvolved skin and even more so in the lesional skin of the psoriatic patient, a process of habituation or tolerance has become manifest with respect to the accumulation of polymorphonuclear leukocytes induced by leukotriene B_4.[1] Such a process might account for the stabilization of the plaque. Therapies such as topical corticosteroids, dithranol, UVB, PUVA, etretinate and methotrexate potentiate this phenomenon.[2,3]

Epidermal proliferation in psoriasis can be explained by an increased recruitment of cycling epidermal cells from the germinative cell population. Na^+/H^+ ATPase has a key role in this recruitment. Amiloride, an inhibitor of this enzyme proved to inhibit the recruitment of cycling cells *in vivo*.[4]

In the near future new therapies will be tested for their efficacy in psoriasis, on the basis of modern insights into the pathogenesis of psoriasis.

REFERENCES

1 Lammers AM. The leukotriene B_4-induced accumulation of polymorphonuclear leukocytes in psoriatic skin. *Thesis, Nijmegen*, 1988.
2 Chang A, van de Kerkhof PCM. Topical application of clobetasol-17-propionate inhibits the intra-epidermal accumulation of polymorphonuclear leukocytes. *Acta Derm Venereol (Stockh)* 1988; **68:** 57–60.
3 Van de Kerkhof PCM. Modulation of leukotriene B_4 induced micropustule formation by antipsoriatic therapy. *Abstracts 17th World Congress of Dermatology*, Berlin 1987, Part I, 155–6.
4 Rijzewijk JJ, Bauer FW, Boezeman JBM *et al.* Recruitment of quiescent (G_0) cells following epidermal injury is initiated by activation of the phosphoinositol cycle. *J Invest Dermatol* 1988; **90:** 44–8.

The paradoxical effects of elastic stockings

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Most investigators agree that elastic stockings are clinically beneficial in patients with venous insufficiency. However, at a theoretical level the interpretation of the physiological effects are controversial.[1,2] The effect of elastic stockings on haemodynamic parameters was analysed in healthy individuals and in patients with moderate venous insufficiency. the effect of elastic stockings, with an ankle pressure of 40 mm Hg, was evaluated with respect to venous pressure and venous volume. The results can be summarized as follows: In normals the calf muscle pump function worsened if the maximum venous pressure drop was regarded as the criterion, whereas an improvement could be shown if volume drop or refill-time was judged. Surprisingly, in patients no improvement of the venous muscle pump function could be shown, as judged by pressure fall. However, the increased volume drop and the increase in the refill-time proved to parallel the clinical improvement.

The explanation for this discrepancy is provided by a non-linear pressure–volume relationship (P_v–V relation), which complicates the interpretation of the parameters for volume and time. It also accounts for the fact that refill-times are equal if pressure and volume are measured simultaneously, whereas the refill-halftimes are not equal.

It should be stressed that the transmural pressure is of utmost importance with respect to the nutritive functioning of the vascular system.

REFERENCES

1 Partsch H. Besserung der venosen Pumpleistung bei chronischer Veneninsuffizienz durch Kompression in Abhängigkeit von Andruck und Material. *VASA* 1984; 13: 58–64.

2 Brakkee AJM, Kuiper JP. Grundlagen der unblutigen Venendruckmessung. In: *Periphere Venendruckmessung*. (May R, Kriessmann A eds). Stuttgart, Thieme Verlag 1978; 86–90. (Praktische Information: 91–7; Standardisierung: 98–102).

Genetic mosaics in dermatology

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The skin is a very suitable organ for the study of human mosaicism. Mosaic phenotypes of the skin mostly display a pattern following Blaschko's lines.

Functional X-chromosome mosaicism. If an X-chromosome mutation interferes with the embryogenesis of the skin, functional mosaicism is seen in a pattern following Blaschko's lines. Either, the X-linked mutation constitutes a lethal factor for male embryos, as has been postulated in X-linked dominant chondrodysplasia punctata, focal dermal hypoplasia and incontinentia pigmenti, or, the mutation is not lethal for males, as observed in X-linked hypohidrotic ectodermal dysplasia. In hemizygous males the sweat glands are almost completely absent. Female carriers show a mosaic distribution of areas devoid of sweat glands, and sweat testing on the back shows the pattern of Blaschko's lines.

Genomic mosaicism. Here, the two populations of cells differ with respect to their genome. The epidermal naevus of the epidermolytic type is apparently due to a non-lethal autosomal mutation. When gonadal mosaicism is present, the underlying mutation can be transmitted to the next generation, although the mosaic pattern of the naevus cannot be transmitted. Instead, the child will suffer from bullous congenital ichthyosiform erythroderma since all cells of the embryo carry the mutation. Lethal autosomal mutations cannot be transmitted, although cells carrying this mutation can survive in close proximity to normal cells. This concept explains the sporadic occurrence of various mosaic phenotypes, such as Schimmelpenning-Feuerstein-Mims syndrome, Proteus syndrome, and McCune-Albright syndrome.[1]

REFERENCES

1 Happle R. Lethal genes surviving by mosaicism: A possible explanation for sporadic birth defects involving the skin. *J Am Acad Dermatol* 1987; 16: 899–906.

Enzymatic diagnosis of congenital ichthyoses

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Phospholipids and glycolipids are essential constituents of plasma membranes. During the process of keratinization the polar groups of these molecules are removed to leave strongly hydrophobic molecules.[1] Enzymes of the lamellar body are crucial in this process.

In genetically-determined disorders of keratinization the primary defect might well be located in one of the enzymes contained in the lamellar body. We have, therefore, developed sensitive micro-assays for certain of these enzymes and carried out partial characterization of their properties.

Preliminary results are as follows:
(1) Six enzymes have been characterized from extracts of scales. They are β-glucosidase, two carboxylic acid esterases (butyrase and palmitase), cathepsin B/L, a phospholipase A_2 and an acid phosphatase. (2) These lamellar-body enzymes seem to differ from those of the classical lysosome, in particular showing a rather higher pH optimum. (3) Scales from patients with erythrodermic autosomal recessive lamellar ichthyosis (EARLI) showed a relatively low β-glucosidase activity, whereas the non-erythrodermic form (NEARLI) possessed little butyrase. Thus the ratio between the butyrase and the glucosidase may be useful for differential diagnosis; in six specimens from EARLI patients this ratio was 13 or higher, and in 10 specimens from NEARLI it was less that 3.

This is the first time that enzymology has been successfully employed as a diagnostic aid in the congenital ichthyoses, and we anticipate that an approach of this sort will help to clarify the nosology of this group of diseases.

REFERENCES

1 Yardley HT, Summerly R. Lipid composition and metabolism in normal and diseased epidermis. In: *The Chemotherapy of Psoriasis* (Baden HP Ed.) Pergamon Press, Oxford, 1984. 55–83.

Skin lesions due to exposure to toxic gases

C.L.A.VAN EYK AND W.A.VAN VLOTEN

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In 1984 and in 1986 two groups of patients (15 in total) were admitted at the University Hospital, Utrecht, for treatment of extensive skin lesions resulting from exposure to toxic gases.

History. Five to seven days before admission to our hospital all patients had been exposed for some hours to gas released by bombs on the battlefield. Acute symptoms of intoxication consisted of nausea, vomiting, dyspnoea, epiphora and photophobia. After 4 h the first skin manifestations, i.e. a burning sensation, appeared.

Dermatological examination. Total skin surface affected varied from 15 to 60%. There were, successively, painful skin defects with superficial and deep necrosis, erosions with crusts and follicular re-epithelialization, flaccid bullae with a positive Nikolsky phenomenon, and erythematous and hyperpigmented lesions.

Physical examination. Conjunctivitis and manifestations of tracheo-bronchitis.

Laboratory data. Transient signs of bone marrow suppression, lowered PO_2 on arterial blood gas analysis.

Histology. Epidermis: fibrin necrosis and vacuolar degeneration of basal cell layers. Dermis: some perivascular lymphohistiocytic infiltration, extravasation of erythrocytes and necrotic tissue.

Treatment and clinical course. Wet compresses of chlorhexidine solution for lesions with exudation and superficial or deep necrosis; silver sulphadiazine ointment for erosions; chloramphenicol ointment for conjunctivitis. Vigorous physiotherapy was given for tracheo-bronchitis. Partial decontamination of the intestinal tract was performed by giving oral amphotericin B, colistin and neomycin. In general, skin lesions healed within 3 to 4 weeks. One of the patients had to be ventilated for distal bronchiolitis. Four months after admission he unexpectedly died of massive lung embolism.

Discussion. The composition of the toxic gases used as offensive weapons to which our patients were exposed is not known. Blistering gases can be divided in three categories: mustard gas and its derivatives, arsenicals and halogenated oximes. These agents have different physical and chemical properties and cause damage to skin, mucous membranes and internal organs by different mechanisms and with variable latency times.[1-3]

REFERENCES

1 Büscher H. *Grun- und Gelbkreuz, spezielle Pathologie und Therapie der Körperschadigungen durch die chemischen Kampfstoffe*. Himmelheber & Co, Hamburg, 1932.

2 Vogt RF, Dannenberg Am, Schofield BH *et al*. Pathogenesis of skin lesions caused by sulfur mustard. *Fundam Appl Toxicol* 1984; 4: 71–83.

3 De Keyser H, Geerts M, Colardyn F, De Bersaques J. Hautschaden durch Einwirkung von Stickstofflost. *Hautarzt* 1986; 37: 467–71.

Immunohistochemical analysis of melanocytic lesions

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Monoclonal antibody technology has enabled the identification of a large number of human melanoma-associated antigens. These antigens can be divided into melanocytic differentiation markers, markers of neoplastic progression and markers of cell functions. Markers of the melanocytic lineage include the high molecular weight antigens, gp 100-7, gp 90-34, P97, GD_3 and S-100. Of these antigens only gp 100-7 is specific for the melanocytic lineage. This antigen, according to immunoelectron microscopy, is localized on the inner side of (pre) melanosomes. Monoclonal antibodies recognizing differentiation markers have been found to be very useful in the diagnosis of poorly differentiated tumours.

Markers of neoplastic progression identify certain stages of melanocytic lesions. Examples of such markers are the K-12 antigens, gp 130, P76, gp89, gp113, HLA-DR and the PAL-M_1 antigen. Although some of these antigens discriminate between naevocellular naevus and primary melanoma, their role in diagnostic pathology has still to be established. However, expression of HLA-DR and the A-1-43 antigens is correlated with an increased risk of metastasis, which is relevant to the prognosis.

Markers associated with cell function of human melanocytes include p97 (transferrin), MACAM-1 (cellular adhesion molecule), and HLA class I and II (immune regulation molecules). This group of markers shows some overlap with the preceding two groups. It is to be expected that more monoclonal antibodies will be developed which may recognize important new determinants relevant to the biology of the human melanocyte.

Immunohistochemical evidence for the malignant potential of congenital melanocytic naevi

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To be published elsewhere.

Topical immunotherapy; indications and results

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In topical immunotherapy, immunological changes are induced by the application of a potent contact allergen. It is used in the palliative treatment of melanoma metastases, and the treatment of recalcitrant warts and alopecia areata.

The concept of antigenic competition might explain the effectiveness of topical immunotherapy in alopecia areata. The typical peribulbar infiltrate might be the result of a cell-mediated immune response to an antigen associated with the matrix. The application of a second allergen might induce the proliferation of suppressor T cells, resulting in a non-specific down-modulation of immune reactions against the hair follicles. Hair regrowth could thus be attributed to changes in the local balance between different T cell subsets. This concept it supported by immunohistochemical findings.

After treatment of 600 patients in Nijmegen, Münster and Homburg/Saar the therapy has been standardized. The contact allergens used are diphenylcycloprogenone (DCP) and squaric acid dibutylester (SADBE). After sensitization with 2% DCP in acetone, weekly elicitation is carried out on one side of the scalp only, with concentrations adjusted according to the eczematous reaction. When regrowth occurs exclusively on the treated side, the whole scalp is treated.

Side-effects of the treatment are itching, erythema, scaling and lymph node enlargement. Other side-effects such as vesicular eczema and erythema multiforme are rare.

Topical immunotherapy combines effectiveness with a low rate of unpleasant side-effects.[1] Furthermore, it can be used a a model for further immunological research.

REFERENCE

1 Happle R, Hausen BM, Wiesner-Menel L. Diphencyprone in the treatment of alopecia areata. *Acta Dermatovenereol (Stockh)* 1983; **63**: 49–52

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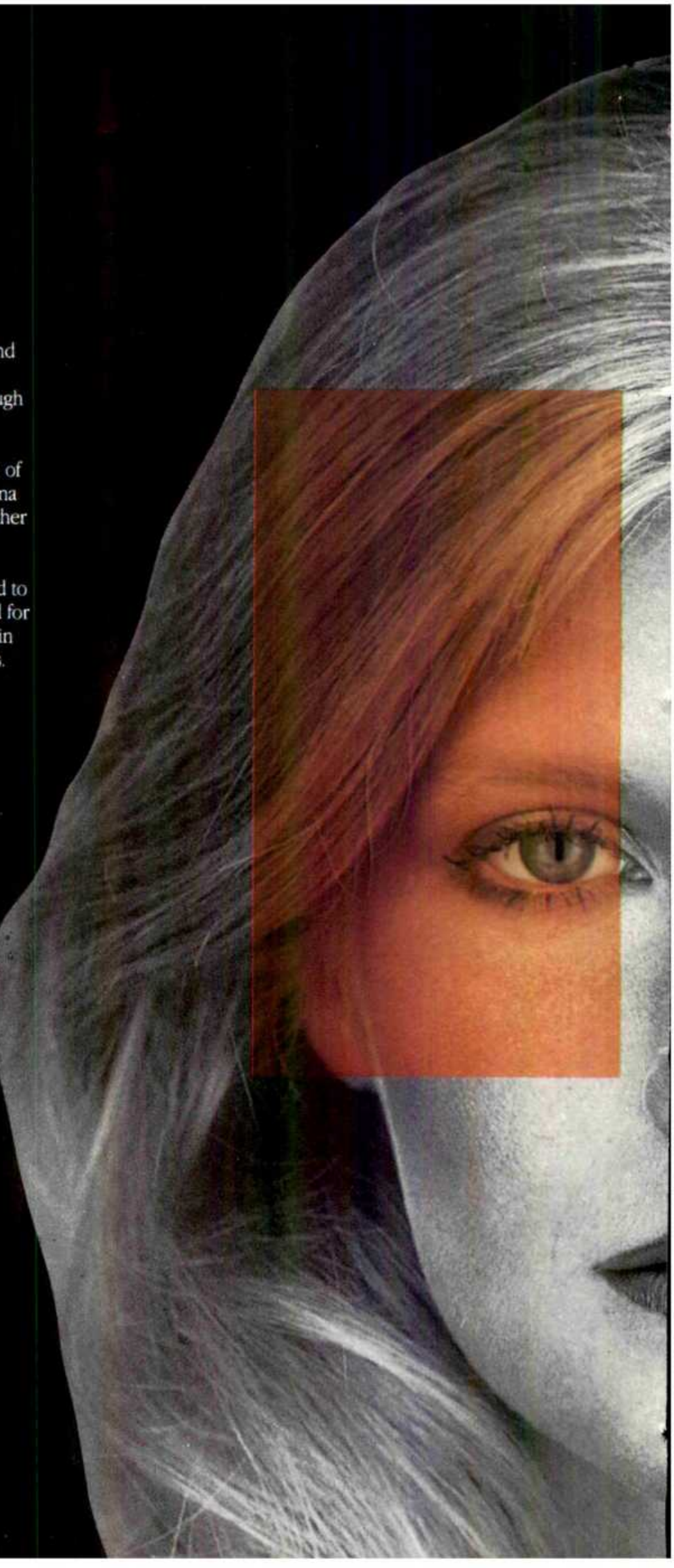

British Journal of Dermatology (1989) **20,** 139–144.

Correspondence

Great toe-nail dystrophy

Sir, Further to the report by Drs Handfield-Jones and Harman of spontaneous improvement in two cases of the great toe nail dystrophy (*British Journal of Dermatology* 1988, **118,** 305) the following review of 42 cases of the dystrophy may be of interest.

There were 22 male and 20 female patients. The first patient was seen in 1978. Most of the patients were young children, 10 of whom were from three sibships. Five were adults, two of whom were fathers of affected children.

In 19 patients bilateral dystrophic great toe-nails, some of which were severely affected, were observed to grow out, leaving clinically normal nails. In two patients a unilateral dystrophy was seen to grow out normally. In one patient with bilateral dystrophy one nail was seen to clear completely; the other improved considerably.

In all patients whose dystrophy cleared this occurred before the age of 10 years; most cleared by 6 years of age. Clearance after the age of 10 years seems unlikely. Improvement begins at the posterior nail fold and once it has begun the whole nail often clears within a year.

At least three patients had other toe-nails affected apart from the great toe-nails. Two patients also had mildly affected finger-nails.

Affected nails show the lesions described by Samman,[1] most typically in infancy and childhood. In adult life partial clearing and the changes naturally associated with ageing can make the diagnosis difficult.

Baran[2] has proposed that lateral deviation of the long axis of the nail is the cause of this dystrophy. Failure of the true nail to grow forwards properly from the matrix into the nail bed from the posterior nail fold may be an equally important factor in the aetiology of the dystrophy. In any case, it appears that in this dystrophy the matrix retains the ability to project a normal plate forwards into the area of the nail bed up to about the age of 10 years.

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REFERENCES

1 Samman PD. Great toe-nail dystrophy. *Clin Exp Dermatol* 1978; **3:** 81–2.
2 Baran R, Bureau H, Sayag J. Congenital malalignment of the big toe-nail. *Clin Exp Dermatol* 1979; **4:** 359–60.

Sir, The cases reported by Hanfield-Jones and Harman (*British Journal of Dermatology* 1988, **118,** 305) are impressive and there is no doubt that in some rare cases, malalignment of the great toe-nails improves and even disappears by itself.

However, the actual frequency of spontaneous realignment is crucial. To date, there are no reported large series. I have seen many patients, less fortunate than those of Drs Hanfield-Jones and Harman, and should like to present two of these. They are adults whose case histories did not parallel the favourable course reported by Drs Hanfield-Jones and Harman.

Case 1. A 69-year-old male patient presented with seborrhoeic dermatitis. Since he knew of our interest in nail conditions he showed us his congenital anomaly involving one of his great toe-nails. The nail plate was greyish, thick, roughly triangular and laterally deviated (Fig. 1). Absence of adherence of the nail to the nail bed enhanced the contrast between the longitudinal borders of the nail bed sited in the axis of the distal phalanx and the malaligned nail.

Case 2. This patient, a 59-year-old female, was seen in our clinic for a painful great toe-nail. It was thickened by a subungual hyperkeratosis and laterally deviated (Fig. 2). This deviation was congenital. We

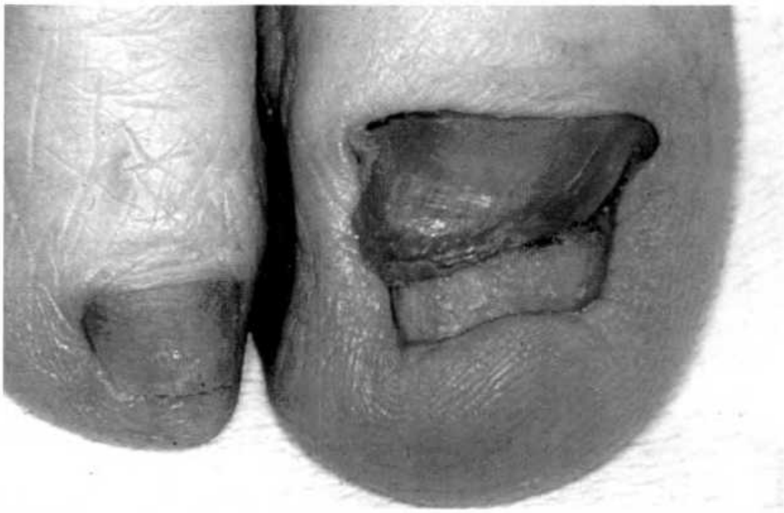

FIGURE 1. Malaligned hypertrophic nail plate in a 69-year-old man, with the nail bed area in the longitudinal axis of the distal phalanx. (First published in *Clinical and Experimental Dermatology* 1983; **8:** 619–23. Reproduced with permission of the publishers.)

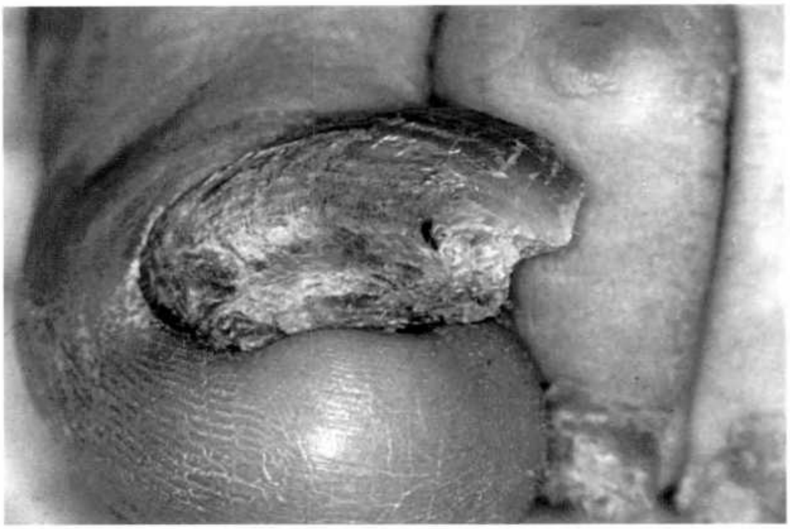

FIGURE 2. Subungual hyperkeratotic laterally malaligned nail.

suggested only abrasion to reduce the thickness of the nail at regular intervals.

In the final analysis, and in the absence of hard criteria, the dermatologist will have to shoulder the responsibility of a difficult decision.

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R. BARAN

Congenital epidermolytic hyperkeratosis associated with multiple malignancies

SIR, We would like to present what we believe to be a unique case of a patient with congenital epidermolytic hyperkeratosis (bullous ichthyosiform erythroderma) who developed squamous cell carcinoma with lymph node metastases, basal cell carcinoma and breast carcinoma.

A 69-year-old woman had developed hard scaly skin over her whole body in early childhood. She had always been of small stature compared to her parents and four sisters who all enjoyed good health. The skin of her feet, knees, hands and elbows was most severely affected (Fig. 1) with a tendency to crack which resulted in severe pain and immobility; even minor movements caused considerable distress. The build-up of keratin caused an embarrassing smell. She also avoided sun exposure as this tended to promote blister formation and swelling of her limbs. There had been no mucosal involvement. A clinical diagnosis of congenital epidermolytic hyperkeratosis was made. Her treatment over the years had included the use of topical emolients and antiseptics, together with periodic courses of antibiotics and ultraviolet light therapy for flare-ups of infection. In addition, she had used many keratolytic and tar preparations as well as courses of retinoids, and oral and topical steroids. She had received two courses of superficial X-ray therapy to her limbs over 10 years previously. Over the last 20 years she had developed a series of skin tumours which had been removed on 15 occasions wtih good wound healing.

Histological examination confirmed the clinical diagnosis of epidermolytic hyperkeratosis characterized by clear spaces surrounding the cells in the thickened stratum spinosum which also showed indistinct cell margins. The oedema between the cells resulted in intra-epidermal bullae. The granular layer was thickened and there was compact hyperkeratosis. The upper dermis contained a patchy chronic inflammatory infiltrate (Fig. 2). In addition, 25 squamous cell carcinomata had developed, 12 were intraepidermal, i.e. *in situ* and 13 showed early invasion of the dermis (Fig. 3); in 1980 metastatic squamous

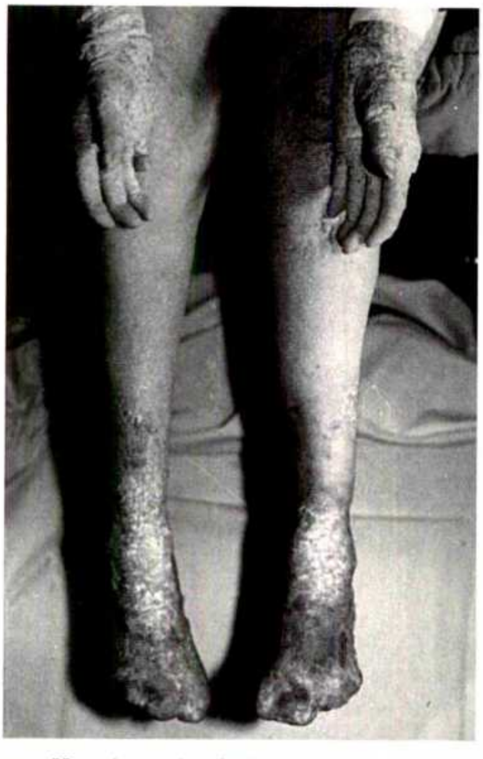

FIGURE 1. Hyperkeratosis affecting the hands, feet and knees.

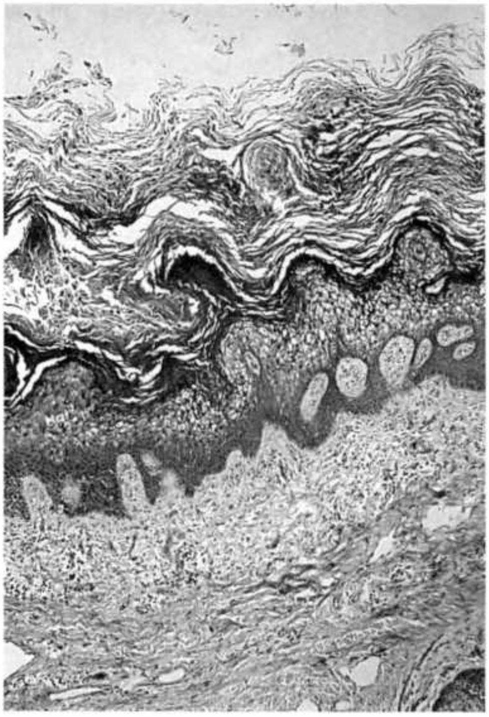

FIGURE 2. Marked lamellar hyperkeratosis with acanthosis. The thickened stratum spinosum shows intercellular oedema and the granular layer is prominent. The dermis is infiltrated by scattered lymphocytes (Original × 78).

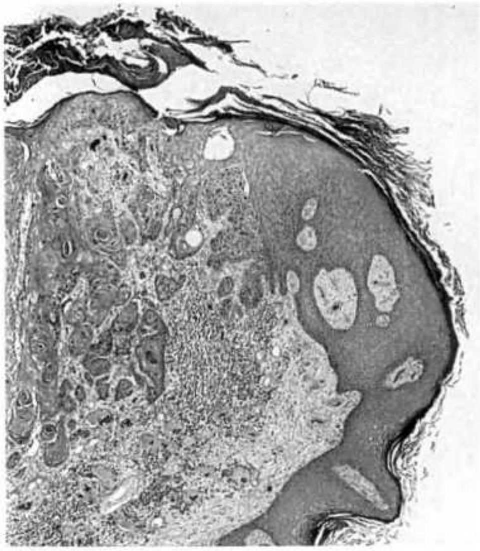

FIGURE 3. On the right of the picture the epidermis is hyperkeratotic and acanthotic with fusion of the rete ridges. An infiltrating squamous cell carcinoma is present on the left of the picture. (Original × 55).

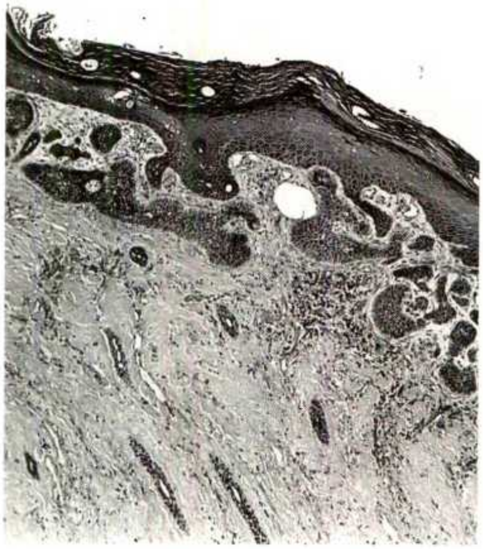

FIGURE 4. A superficial basal cell carcinoma arising from the epidermis. (Original × 95).

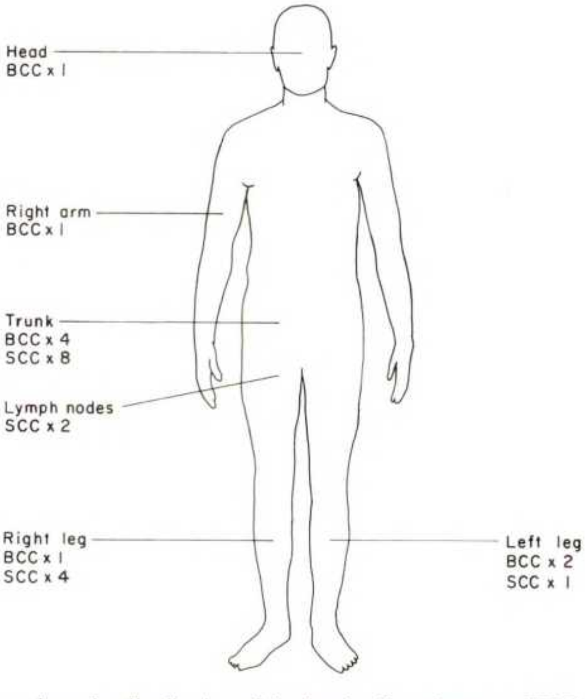

FIGURE 5. Diagram to show the distribution of the basal cell carcinomata (BCC) and the infiltrating squamous cell carcinomata (SCC).

cell carcinoma was found in two lymph nodes of the right superficial inguinal group. Nine basal carcinomata were excised which were superficial and multifocal (Fig. 4). The distribution of the inva skin tumours is illustrated in Figure 5. In 1986 an infiltrating ductal carcinoma was removed from the ri breast.

Acquired ichthyosis is very likely to be associated with malignancy, especially Hodgkin's dise Congenital ichthyosis has been reported in association with ovarian endodermal sinus tumour,[1] carcin of the thyroid[2] and medulloblastoma.[3] There is a case described in the literature in which squamous carcinoma developed in a boy with the KID syndrome (keratitis, congenital ichthyosis and deafness)[4] a report of malignant melanoma developing in two siblings with congenital ichthyosis and xerode pigmentosum.[5] However, we believe that this is the first casae of congenital ichthyosis alone in w multiple squamous cell and basal cell carcinomata developed. A possible explanation for the rarity of finding is the shortened life span of many patients with congenital ichthyosis. The possibility that t tumours may be the result of treatment is difficult to exclude totally; the patient had received nume different treatments over many years. In addition, children of her generation with poor stature sometimes prescribed arsenic containing haematinics. We have no evidence that this was so in her case addition to the multiple skin tumours the patient also developed an infiltrating ductal carcinoma of breast. Casual association is probably the most likely answer to this finding.

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M.A.C.S.COOP
S.BANNE

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We would like to thank Dr M.Garretts, Consultant Dermatologist, and Mr C.I.Orton, Consultant Pla Surgeon for allowing us to present this patient.

REFERENCES

1 Lopez M, Papaldo P, Sciarretta F, Barduagni A. Congenital ichthyosis and endodermal sinus tumor of the ovary ten year old child. *Med Pediatr Oncol* 1983; **11:** 238–41.

2 Ruzicka T, Goerz G, Anton-Lamprecht I. Syndrome of ichthyosis congenita, neurosensory deafness, oligophr dental aplasia, brachydactyly, clinodactyly, accessory cervical ribs and carcinoma of the thyroid. *Dermatologica* 1 **162:** 124–36.

3 Walach N. Congenital ichthyosis and medulloblastoma. *Dermatologica* 1977; **154:** 49–52.

4 Madariaga J, Fromowitz F, Phillips M, Hoover HC. Squamous cell carcinoma in congenital ichthyosis with dea and keratitis. *Cancer* 1986; **57:** 2026–9.

5 Lynch HT, Anderson DE, Smith JL *et al.* Xeroderma pigmentosum, malignant melanoma, and conge ichthyosis. A family study. *Arch Dermatol* 1967; **96:** 625–35.

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microgram(s) (10^{-6}g)	μg	cubic millimetre(s)	mm^3	micrometre(s)	μm
nanogram(s) (10^{-9}g)	ng	millimetre(s)	mm	per cent	%
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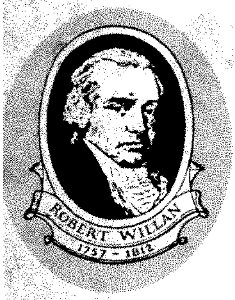

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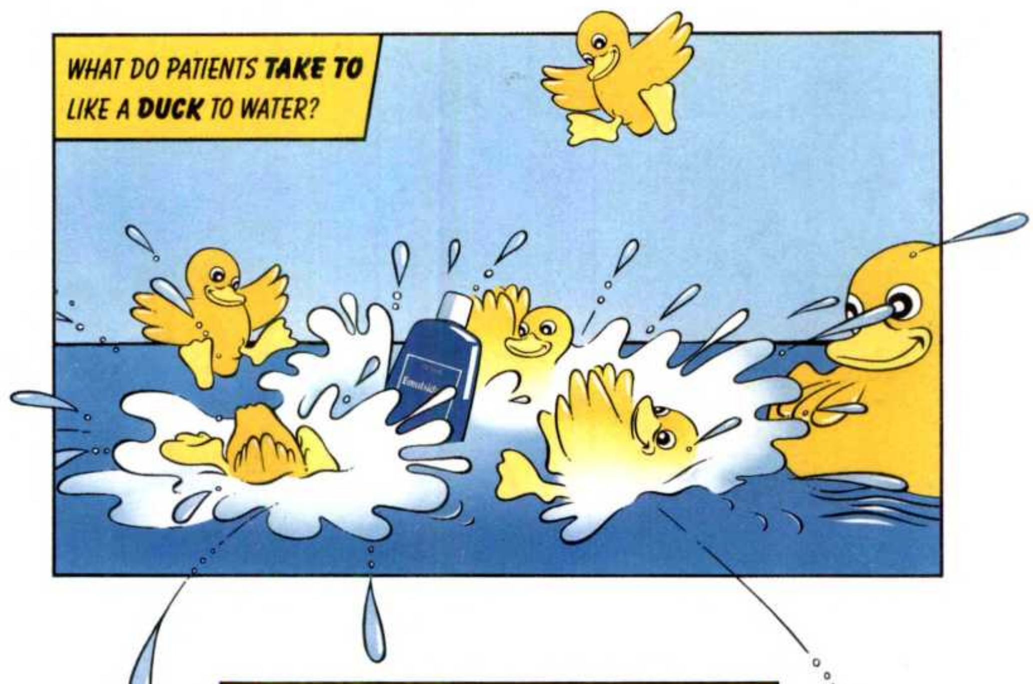
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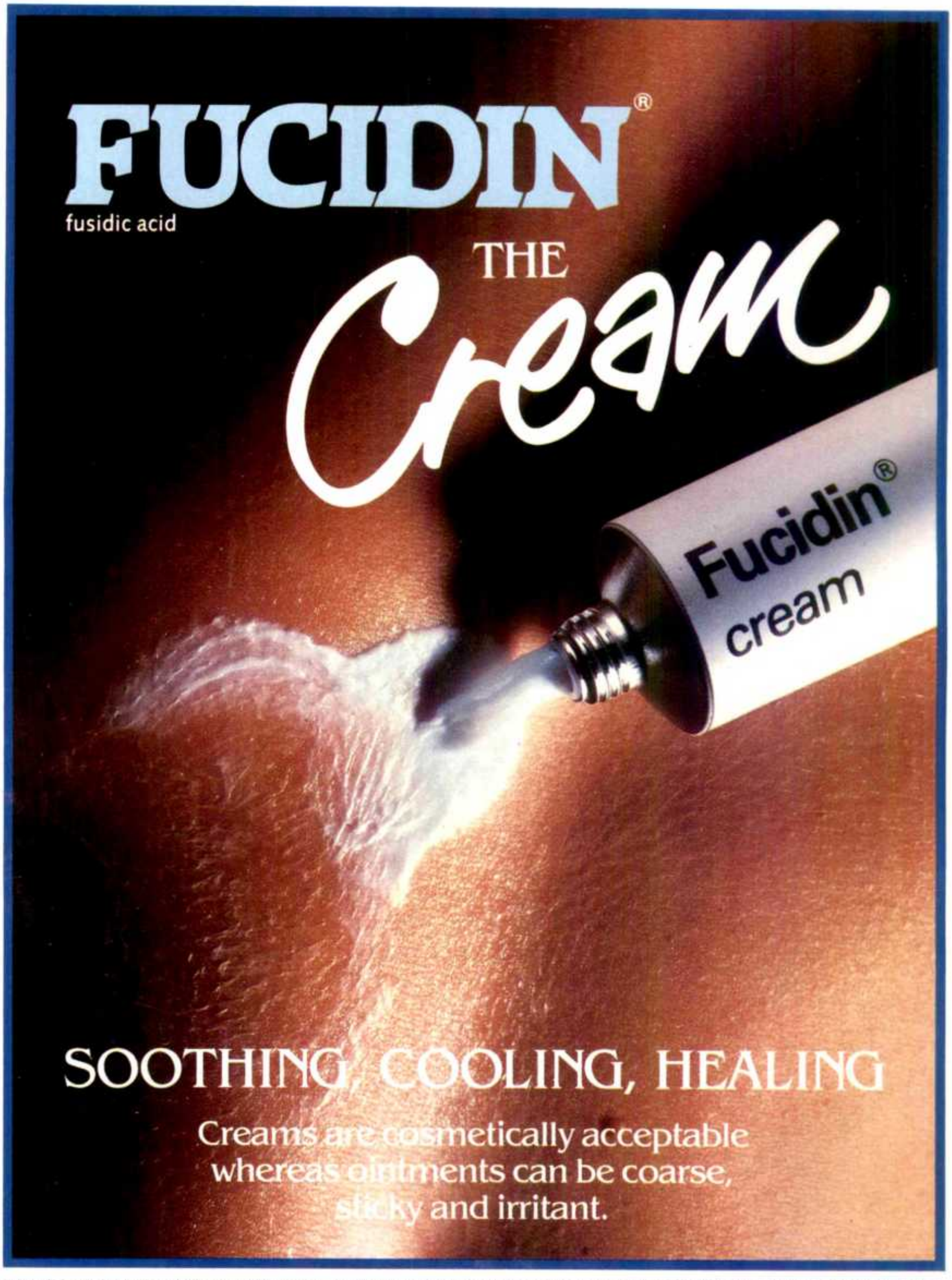
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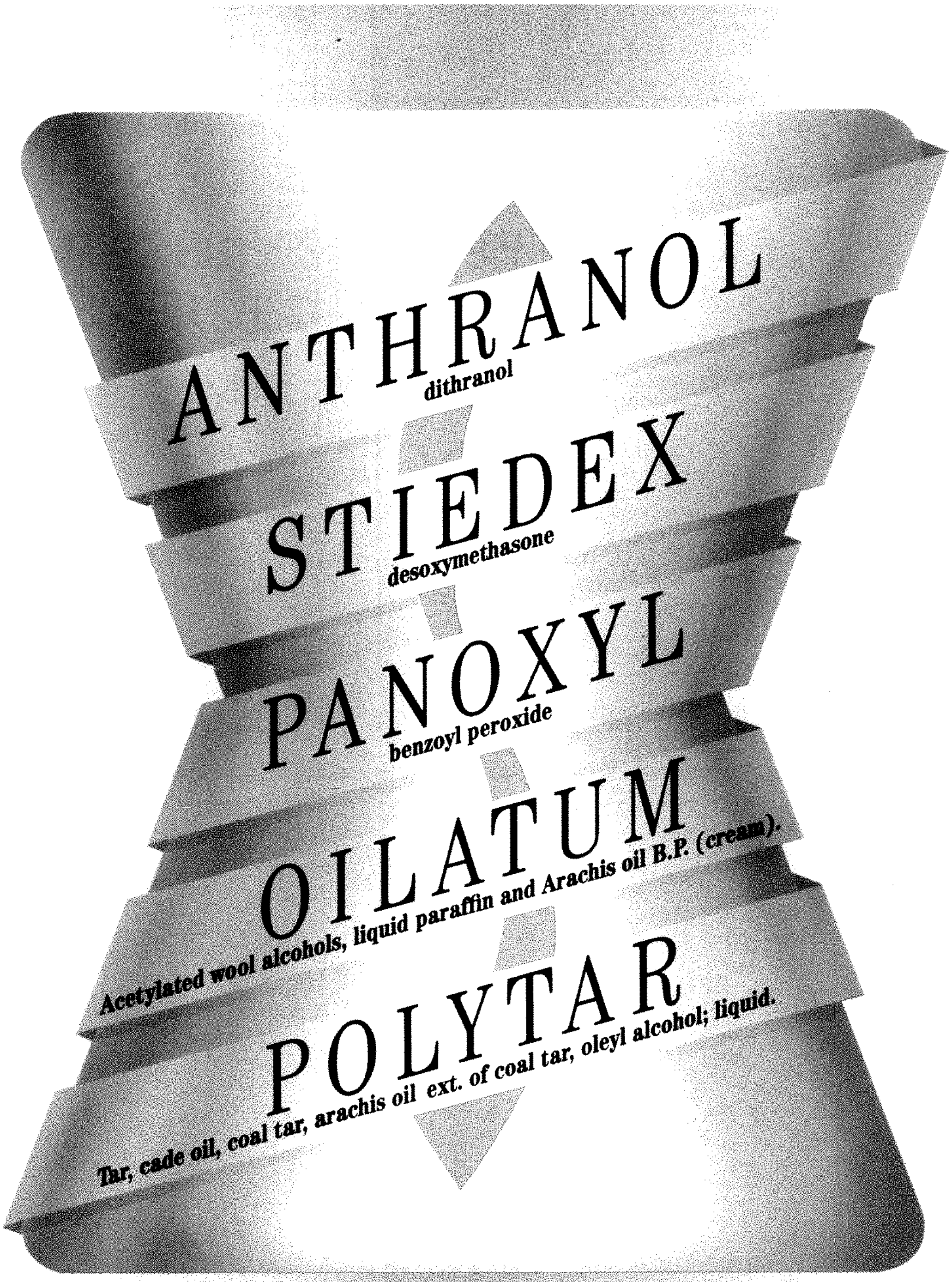

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Dr R. Hay *St John's Hospital for Diseases of the Skin, Lisle Street,*
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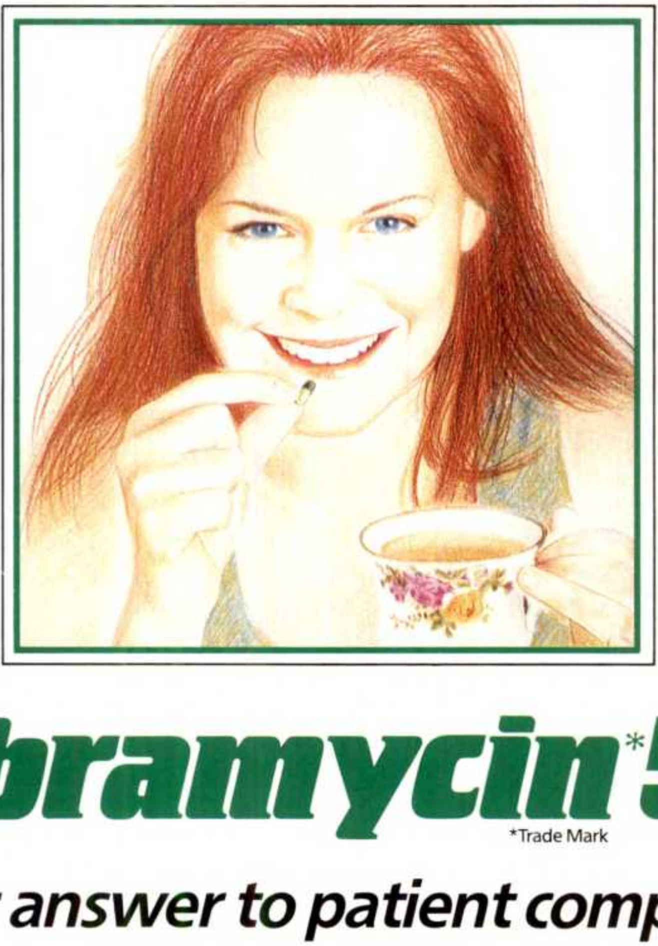

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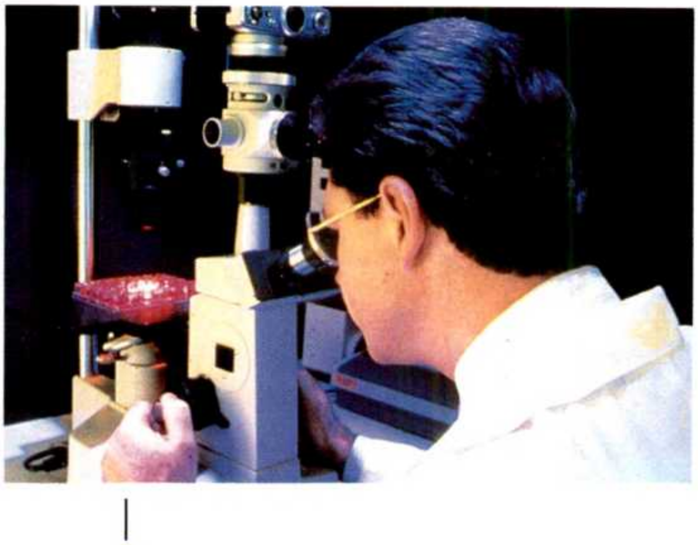

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REFERENCES

1. Mead JF & Fulco AJ 1976 Th unsaturated and polyunsaturate fatty acids in health and diseas C C Thomas, Springfield, USA.
2. Rivers JP Br Med Bull 1981; 3 59-64.
3. Manku MS *et al* Br J Dermat 1984; **110**: 643-648.
4. Ziboh VA & Chapkin RS. Pr Lipid Res 1988; 27: 81-105.
5. Horrobin DF. J Am Ac Dermatol, in press.
6. Stewart JCM *et al* Submitted.
7. Wright S & Burton JL. T Lancet 1982; 2: 1120-1122.
8. Schalin-Karrila *et al.* Br J De matol 1987; **117**: 11-19.
9. Morse PF *et al.* In Press.

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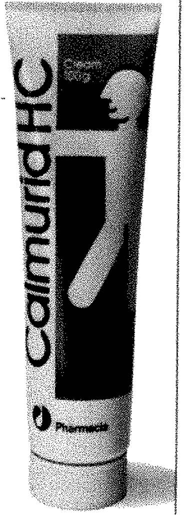

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British Journal of Dermatology (1989) **120**, 329–339.

PUVA therapy decreases HLA-DR$^+$ CD1a$^+$ Langerhans cells and epidermal cell antigen-presenting capacity in human skin, but flow cytometrically-sorted residual HLA-DR$^+$CD1a$^+$ Langerhans cells exhibit normal alloantigen-presenting function

J.ASHWORTH, M.C.KAHAN* AND S.M.BREATHNACH

Department of Medicine (Dermatology), Charing Cross and Westminster Medical School, Fulham Palace Road,
London, U.K. and *Charing Cross Sunley Research Centre, Lurgan Avenue, London, U.K.

Accepted for publication 29 September 1988

SUMMARY

We have investigated the effects of PUVA therapy on human Langerhans cell (LC) immunophenotype and function. Epidermal sheets were obtained from exposed, and control shielded, forearm skin at the end of a course of PUVA therapy, in patients receiving treatment routinely for a variety of dermatoses. PUVA therapy decreased the overall number of HLA-DR$^+$CD1a$^+$ LCs in epidermal sheets, and in epidermal cell (EC) suspensions examined using a fluorescence activated cell sorter (FACS). PUVA therapy also reduced the overall EC allostimulatory capacity in the allogeneic epidermal cell-lymphocyte reaction (ELR), and the capacity of ECs to present tetanus toxoid to, and augment concanavalin A-mediated stimulation of, lymphocytes in the autologous ELR. Depressed allostimulation by ECs from PUVA-treated skin could not be restored by indomethacin (added to block prostaglandin synthesis). The reductions in LC numbers and EC allostimulatory capacity varied according to dose, and time since cessation, of PUVA therapy, and in individual patients were of comparable degree. By contrast, the allostimulatory capacity of residual LCs from PUVA-treated skin (purified using the FACS) did not differ from that of purified control LCs. PUVA-induced suppression of cutaneous immune responses, therefore, results at least in part from an overall impairment of EC antigen-presenting capacity. Residual HLA-DR$^+$CD1a$^+$ LCs in PUVA-treated skin which retain their alloantigen-presenting function may represent a subgroup of PUVA-resistant LCs; alternatively, these cells may be as yet unaffected because they have only recently migrated into the epidermis.

Correspondence to: Dr S.M.Breathnach.

Psoralen plus long wave ultraviolet light (PUVA) therapy is widely used as a treatment for psoriasis and for cutaneous T-cell lymphoma. For this reason, and because PUVA is associated with an increased risk of cutaneous malignancy,[1] there has been much interest in its mechanisms of action. PUVA causes a reversible inhibition of contact sensitization in rodents and humans, which appears to be mediated by antigen-specific suppressor T cells.[2-6] PUVA also inhibits the elicitation phase of allergic contact dermatitis,[3,6,7] but this effect is antigen-non-specific since irritant reactions are also inhibited.[7] PUVA-induced immunosuppression is thought to involve, at least in part, effects on the skin immune system,[8] comprising bone marrow-derived, dendritic antigen-presenting epidermal Langerhans cells (LCs), keratinocytes which secrete a variety of immunoregulatory cytokines, and recirculating T lymphocytes. Reported effects of PUVA on lymphocytes have included a variable decrease in the numbers of the different subsets of circulating T cells,[9-12] and inhibition of *in vitro* alloantigen-stimulated and mitogen-induced lymphocyte proliferation in some but not all studies,[9,12,13-15] perhaps as a result of impaired interleukin 2 production.[16] PUVA also suppresses T lymphocyte migration.[17] In addition to effects on lymphocytes, PUVA results in a dose- and time-dependent reversible decrease in the number of ATPase$^+$, Ia$^+$/HLA-DR$^+$, CD1a$^+$ LCs in rodents and humans.[4,18-26] Exposure to a single dose of PUVA *in vitro* results in a dose-dependent reduction in the alloactivating and antigen-presenting capacity of human epidermal cells (ECs) in the epidermal lymphocyte reaction (ELR).[13,27] Studies involving *in vitro* PUVA therapy of human ECs are somewhat removed from the clinical situation. We have, therefore, investigated the effects of courses of *in vivo* PUVA therapy on the antigen-presenting function of human Langerhans cells, in isolation from potential effects on lymphocytes, in patients receiving treatment routinely for a variety of dermatoses. We report here that although PUVA therapy reduces the number of HLA-DR$^+$CD1a$^+$ LCs and overall epidermal cell (EC) antigen-presenting capacity in the ELR, flow cytometrically purified residual HLA-DR$^+$CD1a$^+$ LCs in PUVA-treated human skin retain normal antigen presenting function.

METHODS

Subjects and PUVA treatment

Patients with a variety of dermatoses routinely undergoing treatment with standard PUVA therapy (0·6 mg/kg 8-methoxypsoralen) were studied; informed consent was obtained from all individuals. Long-wave ultraviolet irradiation (UV-A) was administered with a London Photochemotherapy Apparatus equipped with Phillips TL 85-100W09 UV-A bulbs with a peak emission at 370 nm. Patient details and PUVA therapy are listed in Tables 1 and 2. Subjects received whole body exposure except to the flexor aspect of one forearm, which was shielded from UV-A exposure throughout the treatment course. Epidermal sheets were harvested by the suction-blister method as previously described from uninvolved PUVA-exposed and control shielded forearm sites at varying times after the completion of therapy, and were divided for use either directly in immunofluorescence studies or for processing to obtain suspensions of disaggregated ECs.[28]

Serological reagents

Fluoresceinated OKT6 (FITC-T6) was obtained from Ortho Diagnostic Systems Inc., High Wycombe, Buckinghamshire, U.K., and phycoerythrin-conjugated anti-HLA-DR (PE-DR) from Lab Impex Ltd., Teddington, Middlesex, U.K., and were used at a dilution of 1:20 in Dulbecco's phospate buffered saline (PBS, Gibco U.K. Ltd., Paisley, Scotland). Serum from young rabbits was used as a source of complement.

Preparation of cells

Peripheral blood mononuclear leucocytes (PBMNL) were obtained by centrifugation of heparinized venous blood over a Ficoll–Hypaque (Gibco) density gradient. Purified T cells depleted of HLA-DR$^+$ cells were obtained for use in the autologous ELR by passage of PBM NL suspensions through a nylon wool column followed by treatment with anti-HLA-DR antibody diluted 1 in 20 in PBS for 30 min at 4°C, and then a 1:20 dilution of fresh rabbit serum as a source of complement.

Disaggregated EC suspensions were obtained following incubation of suction-blister roofs with 0·5% trypsin (U.S. Biochemical Co., Cleveland, OH, U.S.A.) in PBS as previously described.[28] The viability of ECs as determined by trypan blue exclusion ranged between 90 and 95%.

Immunofluorescence staining

Epidermal sheets obtained from suction-blister roofs were fixed and stained by a double immunofluorescence method as previously described.[28] FITC-T6 and PE-DR were combined in optimal dilutions (1:20) in PBS containing 1% fetal bovine serum, and incubated with the epidermal sheets for 5 h at 37°C. Epidermal sheets were then washed in PBS, mounted *en face*, and examined using a Leitz EB22 microscope equipped with epifluorescence. The mean number of dendritic LCs per mm^2 staining with fluorescein alone, phycoerythrin alone, or with both fluorochromes was determined by counting five random fields of area 0·1 mm^2 with use of an ocular grid. This number is referred to as the frequency of LC.

EC suspensions were stained by a one-step double immunofluorescence method as previously described.[28] FITC-T6 and PE-DR were combined in optimal dilutions (1:20) in PBS, and incubated with the cells for 30 min at 4°C. The ECs were then washed three times in PBS.

Flow cytometric analysis and sorting

Flow cytometric analysis was performed on EC suspensions which had been stained as above. ECs were resuspended in medium consisting of RPMI 1640 (Gibco U.K. Ltd.) with added antibiotics, 2 M L-glutamine and 15% human AB serum (Sigma Chemical Co., Poole Dorset, U.K.) (complete medium), passed through a sterile nylon mesh filter, and analysed using a Becton Dickinson FACStar equipped with a 5W argon ion laser used at an excitation wavelength of 488 nm and a power of 200 mW. Cells were enumerated relative to green fluorescence, red fluorescence, forward angle light scatter, and right angle light (side) scatter. Using a Hewlett Packard computer and the Consort 30 data analysis program, 10,000 events of list mode correlated data were acquired and analysed.

For functional studies, EC suspensions were sorted at a rate of approximately 1,000 cell/s to obtain the HLA-DR$^+$CD1a$^+$ cells for use in the epidermal lymphocyte reaction. Sorting gates were set on both scatter and fluorescence signals to exclude particulate debris and clumped cells. The sorting procedure was monitored by examining aliquots of the sorted populations, mounted on glass slides under a coverslip, using a Leitz EB22 fluorescence microscope. Populations enriched for HLA-DR$^+$CD1a$^+$LCs contained over 90% of cells staining with both fluorochromes.

Epidermal–lymphocyte reactions

ELR assays were carried out as described.[28] Briefly, for the allogeneic ELR, stimulator ECs from control and PUVA-treated sites were cultured with 5×10^4 unfractionated allogeneic responder PBMNL in complete medium in 96 well round bottom microculture plates

(Nunclon, Gibco UK Ltd.) for 7 days in humidified air containing 5% CO_2. A range of from 1×10^5 to $2{\cdot}5\times10^4$ ECs was added to culture wells in order to obtain an overall profile of allostimulatory capacity, since the concentration of stimulator ECs at which optimal/peak proliferation in the ELR occurs is variable. Wells with responder PBMNL alone were used to determine background counts. In certain experiments, indomethacin 1 μg/ml (Sigma Chemical Co.) was added to the wells.

For the autologous ELR, 5×10^4/well purified T cells, obtained from PBMNL suspensions depleted of $HLA\text{-}DR^+$ cells, were used as the responder cells, and were cultured with varying numbers of autologous ECs. For certain experiments, Concanavalin A (con A; Cambridge Bioscience, Cambridge, U.K., was added to the wells at a final concentration of 80 μg/ml; in other experiments where subjects were known to have received vaccination against tetanus, tetanus toxoid (the gift of Dr M.Feldmann, Charing Cross Sunley Research Centre) was added at a final concentration of 10 μg/ml. Wells containing responder PBMNL alone, to which were added con A or tetanus toxoid, were used to determine background counts.

In further experiments, the allostimulatory capacity of unsorted ECs was compared directly with that of purified $HLA\text{-}DR^+CD1a^+$ LCs, in control and PUVA-treated skin. Unsorted EC suspensions were divided into two aliquots. The first aliquots were used to provide 5×10^4 unsorted stimulator control and PUVA-treated ECs for culture with 5×10^4 allogeneic responder PBMNL. Following analysis and sorting of the remaining aliquots of the original EC suspensions, identical numbers (either 200 or 240) of sorted, $HLA\text{-}DR^+CD1a^+$ enriched control or PUVA-treated ECs (purified LCs) were added to 5×10^4 allogeneic PBMNs per well.

Cultures were pulsed with 1·0 μCi/well of tritiated thymidine (^3H-TdR; Amersham International plc, Amersham, U.K.: specific activity 25 Ci/mmol) for the last 18 h, and were harvested on a cell harvester (Flow Labs, Rickmansworth, Hertfordshire, U.K.). ^3H-TdR incorporation was then assessed by liquid scintillation counting. Results are expressed as the means of triplicate cultures.

RESULTS

Immunofluorescence studies in epidermal sheets

Immunofluorescence examination of epidermal sheets stained with PE-DR and FITC-T6 antibodies revealed a consistent decrease in the frequency of $HLA\text{-}DR^+CD1a^+$ LCs, the degree of which depended on the dose, and time since cessation, of PUVA therapy (Fig. 1 and Table 1). The control frequency of LCs ranged between 199 and 835 cells/mm^2 (mean $\pm$ SEM $= 607 \pm 72$). In certain patients (patients 1 and 8, Table 1) the frequency of LCs remained markedly depressed even at 8–10 days after cessation of PUVA, whilst in the patient who received the lowest cumulative dose (patient 12, Table 1) depression of LC frequency at 8 days after cessation of PUVA was minimal.

In both control and PUVA-treated skin, all LCs expressed both HLA-DR and CD1a antigens; dendritic LCs which were either $HLA\text{-}DR^+CD1a^-$ or $HLA\text{-}DR^-CD1a^+$ were not seen. Focal areas of $HLA\text{-}DR^+$ keratinocytes exhibiting cell surface staining were occasionally seen in control skin in some patients, and were presumably the result of constitutive HLA-DR antigen expression by acrosyringeum; there was a suggestion that focal keratinocyte HLA-DR expression appeared to be enhanced following PUVA therapy (Fig. 1).

PUVA therapy decreases epidermal cell antigen-presenting capacity

The results of a representative experiment, carried out in one individual, showing the effects of

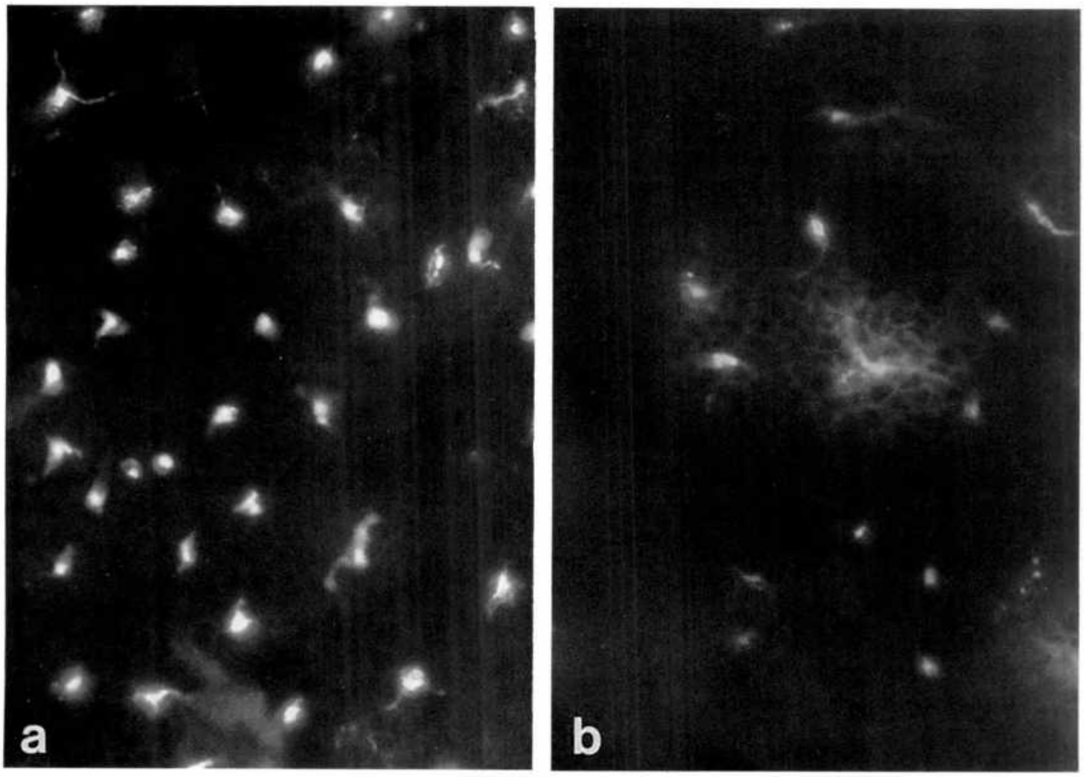

FIGURE 1. Immunofluorescence staining of epidermal sheets with phycoerythrin-conjugated anti-HLA-DR. (a) Control; (b) following PUVA therapy: note reduction in HLA-DR$^+$ LCs, and focal area of keratinocyte HLA-DR expression (× 195).

PUVA therapy on the allogeneic ELR curve are given in Fig. 2. The concentration of control ECs (5×10^4 or 1×10^5 per well) resulting in optimal responder lymphocyte proliferation was determined for each experiment from such ELR curves, for both the allogeneic and the autologous ELR. Results of antigen-presenting capacity of PUVA-treated skin reported here are in general derived by expressing the ^{3}H-TdR incorporation induced by ECs from treated sites at this optimal concentration as a percentage of the ^{3}H-TdR incorporation included by controls ECs at the same concentration. PUVA therapy depressed the alloantigen presenting capacity of ECs in the ELR; the degree of depression varied according to the dose, and time since cessation, of PUVA therapy (Fig. 2, Table 1). There was a correlation in individual patients between the degree of depression of allostimulatory capacity and the degree of reduction in LC density in epidermal sheets (Table 1). The fall in allostimulatory capacity was, in general, most marked in patients who had received PUVA within the last 2 days (patients 3, 10 and 11, Table 1); in the majority (patients 4, 5, 9 and 12, Table 1), but not all (patients 2 and 8, Table 1) of the patients there was a trend for the EC allostimulatory capacity to return towards normal from 7 to 14 days after cessation of PUVA therapy.

Addition of indomethacin to the culture medium did not restore the relative decrease in allostimulatory capacity of ECs from PUVA-treated sites (data not shown), indicating that this decrease was not the result of the production of inhibitory prostaglandins by ECs during the culture period.

TABLE 1. Patient details, enumeration of HLA-DR$^+$CD1a$^+$ LCs in epidermal sheets, and epidermal cell allostimulatory capacity in PUVA-treated skin

Patient	Diagnosis	Skin type	Total dose (J/cm^2)	No. of treatment	Last dose (J/cm^2)	Days since last PUVA	HLA-DR$^+$CD1a$^+$ LCs as % of control*	EC allostimulatory capacity as % of control
1	Psoriasis	III	59·7	13	5·9	8	23	47
2	Psoriasis	II	51·0	14	4·8	7	ND†	19
3	Psoriasis	II	49·0	16	5·7	1	24	10
4	Mycosis fungoides	IV	47·0	11	4·3	10	59	74
5	Psoriasis	III	41·9	12	6·4	14	ND	99
6	Psoriasis	III	40·0	12	4·5	3	ND	45
7	Polymorphic light eruption	II	35·0	12	2·6	1	ND	49
8	Psoriasis	I	33·0	11	3·5	10	17	30
9	Psoriasis	II	31·6	9	4·0	7	61	77
10	Psoriasis	I	24·0	13	5·5	2	32	23
11	Psoriasis	I	22·8	17	1·0	1	ND	34
12	Vitiligo	I	10·7	9	1·5	8	89	98

* In epidermal sheet preparations.

† Not done.

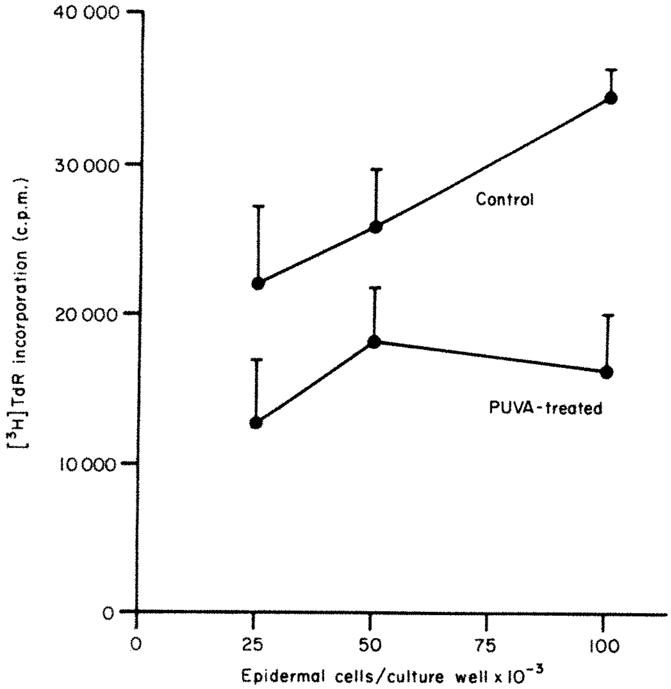

FIGURE 2. Effect of PUVA therapy on the allostimulatory capacity of ECs in the allogeneic ELR; representative experiment showing ELR curves with results expressed as mean ± SEM of triplicate cultures.

TABLE 2. Patient details, enumeration of HLA-DR$^+$CD1a$^+$ LCs in epidermal sheets, and epidermal cell antigen-presenting capacity in the autologous ELR in PUVA-treated skin

Patients	Diagnosis	Skin type	Total dose (J/cm^2)	No. of treatments	Last dose (J/cm^2)	Days since last PUVA	HLA-DR$^+$ CD1a$^+$ LCs as % of control*	EC antigen-presenting capacity as % of control	
								Con A†	TT‡
13	Psoriasis	II	29·7	11	3·9	4	18	63	80
14	Psoriasis	III	16·1	9	2.9	4	16	23	8
15	Psoriasis	II	29·5	9	4·9	2	25	10	59

* In epidermal sheet preparations.
† Conconavalin A.
‡ Tetanus toxoid.

PUVA therapy also reduced the capacity of ECs to induce Langerhans cell-dependent augmentation of con A-stimulated lymphocyte proliferation, and to present tetanus toxoid to sensitized autologous lymphocytes, in the autologous ELR (Table 2).

FACS analysis of EC suspensions from control and PUVA-treated skin

Flow cytometric analysis revealed that PUVA therapy reduced the overall percentage of HLA-

DR$^+$CD1a$^+$LCs in unsorted EC suspensions from 2·80% to 0·51%, and from 0·92% to 0·64 respectively in the two patients investigated (patients 6 and 11, Table 1). HLA-DR$^+$CD1a$^-$ a HLA-DR$^-$CD1a$^+$ECs were not demonstrated in control or PUVA-treated skin in eit patient.

Residual HLA-DR$^+$CD1a$^+$ LCs in PUVA-treated skin demonstrate normal alloantig presenting capacity

Experiments with skin from these two patients (patients 6 and 11, Table 1) confirmed that t alloantigen-presenting capacity of unsorted ECs from PUVA-treated sites was decreas compared with that of control ECs (Table 3). In parallel simultaneous experiments, aliqu taken from the identical unsorted EC suspensions were subjected to flow cytometric sorting a enriched for HLA-DR$^+$CD1a$^+$ ECs, which on examination were seen to consist of 90% L In contrast to the findings of depressed allostimulation by unsorted EC suspensions, in bo patients the allostimulatory capacity of such purified HLA-DR$^+$CD1a$^+$ LCs from PUV treated skin was essentially unchanged from that of equivalent numbers of control purifi HLA-DR$^+$CD1a$^+$ LCs (Table 3).

TABLE 3. Alloantigen presenting capacity of all epidermal cells, and of flow cytometrically-enriched HLA-DR$^+$CD1a$^+$ LCs, from control and PUVA-treated sites

	^{3}H-Thymidine incorporation: mean CPM ± SEM			
	Unsorted epidermal cells†		HLA-DR$^+$CD1a$^+$ Enriched epidermal cells‡	
Patient*	Control	PUVA-treated	Control	PUVA-treated
6	17,785 ± 3,592	8,034 ± 3,608	8,032 ± 3,375	8,350 ± 1,992
11	28,147 ± 1,502	9,551 ± 2,188	7,813 ± 1,607	14,434 ± 1,063

* Patient details are as given in Table 1.

† 5 × 10^4 unsorted stimulator ECs were added to 5 × 10^4 allogeneic responder PBMNL per well.

‡ The following number of sorted HLA-DR$^+$CD1a$^+$-enriched ECs (90% LCs) were added to 5 × 10^4 allogeneic responder PBMNL per well: for patient 6:200; for patient 11:240.

DISCUSSION

As a necessary preliminary step in the investigation of PUVA effects on LCs, we confirm previous reports that PUVA therapy reduces the number of epidermal HLA-DR$^+$CD1a$^+$L as determined by examination of epidermal sheets[4,18–25] and FACS analysis of EC suspe sions.[26] It is still unknown whether PUVA simply modulates surface marker expression LCs, or whether LCs are destroyed or encouraged to migrate away from the epidermis followi PUVA therapy. The reappearance of Ia$^+$/HLA-DR$^+$ LCs following cessation of PU therapy[22] could be explained either by replenishment from a circulating pool derived from the bone marrow, or by re-expression of Ia/HLA-DR antigen. Electron microscopy has not entirely resolved this issue; in man it has been reported that PUVA decreases the number of ultrastructurally identifiable epidermal LCs,[21-23] whereas in mice numerous epidermal LCs were identified with the electron microscope despite a PUVA-induced reduction in Ia^+ cells.[25] Our finding of increased focal keratinocyte HLA-DR antigen expression in PUVA-treated skin in some patients would be compatible either with shedding of the HLA-DR antigen from damaged LCs or with increased keratinocyte synthesis of HLA-DR.

We have extended previous morphological studies on the effects of *in vivo* PUVA therapy in man by demonstrating that the PUVA-induced reduction in $HLA\text{-}DR^+CD1a^+$LCs is associated with a fall in the overall alloantigen-presenting capacity of ECs, as well as in the capacity of ECs to produce mitogen-stimulated and antigen-specific T lymphocyte proliferation. It has been reported that prostaglandins may be involved in the inhibition of EC antigen presentation and of contact hypersensitivity following ultraviolet B light irradiation.[29] PUVA-induced inhibition of EC antigen-presenting function in our study was not, however, the result of synthesis of prostaglandin E_2 by ECs, since allostimulation was not enhanced by addition of indomethacin to the ELR culture medium. The reduction in LC numbers and the overall EC antigen-presenting capacity varied with the dose, and time since cessation, of PUVA, and there was a correlation between the degree of reduction in LC numbers and in EC antigen-presenting function in individual patients. There was a trend for both LC numbers and overall EC allostimulatory capacity to return towards normal with increasing time since cessation of PUVA, in line with the reported time-dependent recovery from PUVA-induced diminution of delayed hypersensitivity responses *in vivo*.[6]

The experimental design of this study was such that it enabled us to analyse effects of *in vivo* PUVA on LC function in isolation, and to deliberately exclude consideration of PUVA effects on lymphocytes. Therefore, we have been able to demonstrate that the immunosuppressive effect of PUVA therapy would seem to involve at least in part a reversible decrease in overall epidermal antigen presenting capacity consequent upon a fall in the number of functioning LCs. It should, of course, be appreciated that *in vivo* PUVA may affect cutaneous immune responses not only by impairing local LC-dependent antigen presentation in the skin as shown here, but also systemically by affecting T lymphocyte function.[9-17]

Although the overall EC antigen-presenting capacity was reduced following PUVA therapy, it is of interest that flow cytometrically purified residual $HLA\text{-}DR^+CD1a^+$ epidermal LCs in PUVA-treated human skin retained normal alloantigen presenting function. The nature of these residual $HLA\text{-}DR^+CD1a^+$ LCs is uncertain. We have recently shown that, following topical steroid therapy in man, residual $HLA\text{-}DR^+CD1a^+$ epidermal LCs also exhibit normal alloantigen presenting function, despite a reduction in the overall EC allostimulatory capacity and the number of $HLA\text{-}DR^+CD1a^+$ LCs.[30] It may be that functionally normal residual $HLA\text{-}DR^+CD1a^+$ LCs in both PUVA-treated and steroid-treated human skin represent a population of LCs which are as yet unaffected because they have only recently immigrated into the epidermis from the circulating pool. Alternatively, the fact that a proportion of LCs retain their surface markers and their antigen-presenting function after both topical steroid and PUVA therapy may indicate that there exist subgroups of LCs with differing susceptibility to the effects of these immunosuppressive treatments.

ACKNOWLEDGMENTS

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REFERENCES

1 Stern RS, Laird N, Melski JM *et al.* Cutaneous squamous cell carcinomas in patients treated with PUVA. *N Engl J Med* 1984; **310:** 1156–61.

2 Strauss GH, Greaves M, Price M *et al.* Inhibition of delayed hypersensitivity reaction in skin (DNCB test) by 8-methoxypsoralen photochemotherapy. *Lancet* 1980; **ii:** 556–9.

3 Moss C, Friedmann PS, Shuster S. Impaired contact hypersensitivity in untreated psoriasis and the effects of photochemotherapy and dithranol/UV-B. *Br J Dermatol* 1981; **105:** 503–8.

4 Lynch DH, Gurish MF, Daynes RA. Relationship between epidermal Langerhans cell density ATPase activity and the induction of contact hypersensitivity. *J Immunol* 1981; **126:** 1892–7.

5 Kripke ML, Morison WL, Parrish JA. Systemic suppression of contact hypersensitivity in mice by psoralen plus UVA radiation (PUVA). *J Invest Dermatol* 1983; **81:** 87–92.

6 White SI, Friedmann PS, Moss C, Simpson JM. Recovery of cutaneous immune responsiveness after PUVA therapy. *Br J Dermatol* 1988; **118:** 403–7.

7 Thorvaldsen J, Volden G. PUVA-induced diminution of contact allergic and irritant skin reactions. *Clin Exp Dermatol* 1980; **5:** 43–6.

8 Breathnach SM, Katz SI. Cell-mediated immunity in cutaneous disease. *Human Pathol* 1985; **17:** 161–7.

9 Morison WL, Parrish JA, Bloch KJ, Krugler JL. *In vivo* effects of PUVA on lymphocyte function. *Br J Dermatol* 1981; **104:** 405–13.

10 Moscicki RA, Morison WL, Parrish JA *et al.* Reduction of the fraction of circulating helper–inducer T cells identified by monoclonal antibodies in psoriatic patients treated with long-term psoralen/ultraviolet-A radiation (PUVA). *J Invest Dermatol* 1982; **79:** 205–8.

11 Larussa FM, Larocca LM, Venier A *et al.* Distribution of circulating mononuclear cells in short-term PUVA-treated psoriatic patients and healthy subjects. *Acta Derm Venereol* 1986; **66:** 398–403.

12 Fraki JE, Eskola J, Hopsu-Havu VK. Effect of 8-methoxypsoralen plus UVA (PUVA) on lymphocyte transformation and T cells in psoriatic patients. *Br J Dermatol* 1979; **100:** 543–50.

13 Morhenn VB, Benike CJ, Engleman EG. Inhibition of cell mediated immune responses by 8 methoxypsoralen and long-wave ultraviolet light: a possible explanation for the clinical effects of photoactivated psoralen *J Invest Dermatol* 1980; **75:** 249–252.

14 Scherer R, Kern B, Braun-Falco O. UVA-induced inhibition of proliferation of PHA-stimulated lymphocytes from humans treated with 8-methoxypsoralen. *Br J Dermatol* 1977, **97:** 519–28.

15 Kruger JP, Christophers E, Schlaak M. Dose-effects of 8-methoxypsoralen and UVA in cultured human lymphocytes. *Br J Dermatol* 1978; **98:** 141–4

16 Okamoto H, Horio T, Maeda M. Alteration of lymphocyte functions by 8-methoxypsoralen and long-wave ultraviolet radiation. II. The effect of *in vivo* PUVA on IL-2 production. *J Invest Dermatol* 1987; **89:** 24–6.

17 Okamoto H, Takigawa M, Horio T Alteration of lymphocyte functions by 8-methoxypsoralen and long-wave ultraviolet radiation. I. Suppressive effects of PUVA on T lymphocyte migration *in vitro*. *J Invest Dermatol* 1985; **84:** 203–5.

18 Okamoto H, Horio T. The effect of 8-methoxypsoralen and long-wave ultraviolet light on Langerhans cells. *J Invest Dermatol* 1981; **77:** 345–6.

19 Friedmann PS. Disappearance of epidermal Langerhans cells during PUVA therapy. *Br J Dermatol* 1981; **105:** 219–21.

20 Tjernlund U, Juhlin L Effect of UV-irradiation on immunological and histochemical markers of Langerhans cells in normal appearing skin of psoriatic patients. *Arch Dermatol Res* 1982; **272:** 171–6

21 Ree K. Reduction of Langerhans cells in human epidermis during PUVA therapy: a morphometric study. *J Invest Dermatol* 1982; **78:** 488–92.

22 Friedmann PS, Ford G, Ross J, Diffey BL Reappearance of epidermal Langerhans cells after PUVA therapy. *Br J Dermatol* 1983; **109:** 301–7.

23 Koulu L, Soderstrom K-O, Jansen CT. Relation of antipsoriatic and Langerhans cell depleting effects of systemic psoralen photochemotherapy: a clinical, enzyme histochemical, and electron microscopic study. *J Invest Dermatol* 1984; **82:** 591–3.

24 Czernielewski J, Juhlin L, Shroot S, Brun P. Langerhans cells in patients with psoriasis: effect of treatment with PUVA, PUVA bath, etretinate and anthralin. *Acta Derm Venereol* 1985; **65:** 97–101.

25 Stingl G, Aberer W, Romani N *et al.* Effects of short-wave UV and PUVA treatment on structure and phenotype of bone marrow-derived dendritic cells of the mouse epidermis. *Curr Probl Dermatol* 1986; **15:** 195–204.

26 Gommans JM, Van Hezik SJTM, Van Huyste BEWL. Flow cytometric quantification of T6-positive cells in psoriatic epidermis after PUVA and methotrexate therapy. *Br J Dermatol* 1987; **116:** 661–6.

27 Mørk N-J, Gaudernack G, Braathen LR. Effect of UVA and PUVA on alloactivating and antigen-presenting capacity of human epidermal Langerhans cells. *Photodermatol* 1986; **4:** 66–72.

28 Ashworth J, Booker J, Breathnach SM. Effect of topical corticosteroid therapy on Langerhans cell function in human skin. *Br J Dermatol* 1988; **118:** 457–69.

29 Jun B-D, Roberts LK, Cho B-H *et al.* Parallel recovery of epidermal antigen-presenting cell activity and contact hypersensitivity responses in mice exposed to ultraviolet irradiation: the role of a prostaglandin-dependent mechanism. *J Invest Dermatol* 1988; **90:** 311–6.

30 Ashworth J, Kahan MC, Breathnach SM. Flow cytometrically-sorted residual HLA-DR$^+$T6$^+$ Langerhans cells in topical steroid-treated human skin express normal amounts of HLA-DR and CD1a/T6 antigens and exhibit normal alloantigen-presenting capacity. *J Invest Dermatol* (in press.)

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Reduced density and morphologic alteration of Ia and ADPase positive Langerhans cells after low-protein diet

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SUMMARY

This study examined the effects of low-protein diet on the population density, morphology and histochemical characteristics of Langerhans cells. Weaned at 18 days old, BALB/c mice were divided into two groups: one group received a high-protein diet (20% casein) and the other was fed an isocaloric low-protein diet (caesin 4%). After 14 days, the mice were killed and the skin of the ears was removed for investigation. Langerhans cells were visualized using ADPase and anti-Ia immunoperoxidase techniques. In protein malnourished mice, the density of ADPase and Ia-positive Langerhans cells was significantly reduced, while morphometric assessment of their cross-sectional area showed a significant reduction of total cell area, cell body area and degree of arborization. Quantitative cytophotometric analysis revealed a reduction of ADPase ectoenzyme activity and decreased concentration of membrane Ia antigen. We postulate that these changes affect Langerhans cell functions, and in turn influence the immune mechanisms in the skin.

Clinical and experimental studies show that protein malnutrition is associated with impairment of cutaneous delayed hypersensitivity and defence mechanisms against infection.[1–3] In addition, several investigators have demonstrated that the number of phagocytic cells in protein malnourished mice is reduced, while the capacity of antigen-pulsed peritoneal macrophages to trigger the proliferation of antigen-primed T cells *in vitro* is severely impaired.[4,5]

Langerhans cells share many features of cells of the mononuclear phagocyte system,[6,7] and have been classified by some investigators as representatives of that system in the skin.[7,8] Because low-protein diet influences the number and function of macrophages,[4,5] it is conceivable that protein malnutrition would also affect Langerhans cells. To understand more

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about the effects of protein malnutrition on the immune mechanisms in the skin, we conducted experiments to elucidate the effects of low-protein diet on the Langerhans cells. The population density, morphometry, ADPase ectoenzyme activity, and Ia antigen concentration of Langerhans cells were determined.

METHODS

Animals

The BALB/c litters used for this study were received from the Animal Resources Centre of Western Australia. The mice were weaned at the age of 18 days, and then were divided into two groups. Each group had the same sex distribution. A total of 46 mice were used in this experiment.

Diet

The experimental group was fed a low-protein diet (4% casein), while the control group received a high (normal) protein diet (20% casein). Both diets contained adequate levels of vitamins and minerals.[9] To compensate for the caloric loss, corn flour was added to the low-protein diet.[9] Food and water were available *ad libitum*. After 14 days on these diets the mice were killed.

Epidermal separation

The ears were excised and the hair was removed using a hair removing cream (Veet, Reckitt Tioletrie, Hull, England). The skin was then cut into approximately 4 × 4 mm, and soaked in 20 mMol Na-EDTA in phosphate-buffered saline (PBS) for 2 h at 37°C.[10] The epidermis was separated using fine forceps under a dissecting stereoscope and the epidermal sheets were kept flat by floating them dermal side down on the various solutions used in subsequent processing. Epidermal sheets for ADPase staining were immediately fixed in cold (4°C) 2% cacodylate-buffered formalin, while those for immunoperoxidase staining were floated on tris-buffered saline (TBS) pH 7·6.

ADPase staining

Selective enzyme staining for Langerhans cells was performed using a modification of the ADPase technique described by Chaker *et al.*[11] The epidermal sheets were treated as follows: fixed in cold (4°C) 2% cacodylate-buffered formalin for 60 min; washed in PBS with continual stirring; incubated at 37°C for 35 min in a freshly prepared and filtered substrate containing 42 ml Trismal buffer (pH 7·3), 5 ml of 5% $MgSO_4$, 10 mg ADP (Sigma Chemical Co., A 6521, St. Louis, Mo, U.S.A.) and 3 ml of 2% $Pb(NO_3)_2$; washed in PBS with continual magnetic stirring. They were incubated in dilute ammonium sulphide solution (2 drops of concentrated ammonium polysulphide in 50 ml distilled water) at room temperature for 2 min; and rewashed in PBS. Finally, the epidermal sheets were placed on glass slides dermal side down, air dried, and mounted under glass coverslips in DPX. Epidermal sheets from mice on high and low-protein diets were stained at the same time in the same solutions, permitting their comparison.

Immunoperoxidase staining

The technique employed was a modification of the avidin–biotin method.[12] The epidermal sheets were fixed in acetone for 20 minutes at 4°C. Endogenous peroxidase activity was blocked with 0·3% hydrogen peroxide for 3 min. The epidermal sheets were subsequently incubated for

3 h in a 1:2000 dilution of anti-Ia monoclonal antibody (provided by Dr P.Price of the Department of Microbiology, University of Western Australia); 1 h in a 1:100 dilution of biotinylated sheep anti-mouse Ig (Amersham), and 1 h in a 1:200 dilution of streptavidin horseradish–peroxidase conjugate (Amersham). Between each step and the various incubations, the epidermal sheets were rinsed in TBS with continual magnetic stirring. The reaction product was developed by incubating the epidermal sheets in 0·06% 3′,3′-diaminobenzidine tetrahydrochloride (DAB) and 0·03% hydrogen peroxide in TBS for 4 min. The brown staining product was intensified by floating the epidermal sheets in 0·5% $CuSO_4$ in saline for 2 min. Finally, the epidermal sheets were placed on glass slides dermal side down, air dried, and mounted under glass coverslips in DPX. Again, epidermal sheets from mice on high and low-protein diet were stained at the same time in the same solutions, permitting their comparison.

Langerhans cell enumeration and morphometric analysis

Because it was impossible to obtain three-dimensional measurements, we measured the cross-sectional area of the profile of whole cells and that of the cell bodies. Morphometric assessment was carried out using the MOP-videoplan image anlaysis system (Zeiss, West Germany). The slides were projected onto a graphics tablet. The cell profiles displayed were then outlined manually using a stylus. The whole profile of the cell was first outlined and the total cross-sectional cell area was computed. The cross-sectional cell body area was then measured and the area occupied by the arborized cytoplasm was calculated by subtracting the cross-sectional cell body area from cross-sectional total cell area. Langerhans cell density was also computed on the same apparatus by counting the number of cell bodies in a calibrated unit area. Data expressed as LC number/mm^2 were obtained by linking the apparatus to an appropriately programmed computer.

Quantatitive cytophotometry

Scanning cytophotometry was performed using a Zeiss cytoscan microscope interfaced to a PDP 11/03 computer. The cells were scanned using a step size of 0·5 μm. All measurements were made using monochromatic light of wavelength 520 nm. Scanning and computations were achieved with the HIDACSYS programs of van der Ploeg *et al.*[13] Because there was a significant difference in the total cell area between the two groups, the mean absorbance per unit area rather than the total absorbance of each individual cell was computed.

Statistical analysis

Data were expressed as means ± SE (standard error of the mean). Two-tailed Students *t*-test was used to investigate differences between sample mean values when necessary. The cell density, mean absorbance values of ADPase as well as immunoperoxidase reaction products, total cell area, and cell body area, in high and low-protein diet groups were compared. A value of $P < 0·05$ was taken to indicate a significant difference.

RESULTS

Langerhans cell density

Langerhans cell density in the skin of mice on a low-protein diet was decreased (Fig. 1). Demonstration of LCs either using ADPase or immunoperoxidase techniques gave essentially similar results; both revealed a significant reduction of Langerhans cell density ($P < 0·001$) in those animals on the low-protein diet (Table 1).

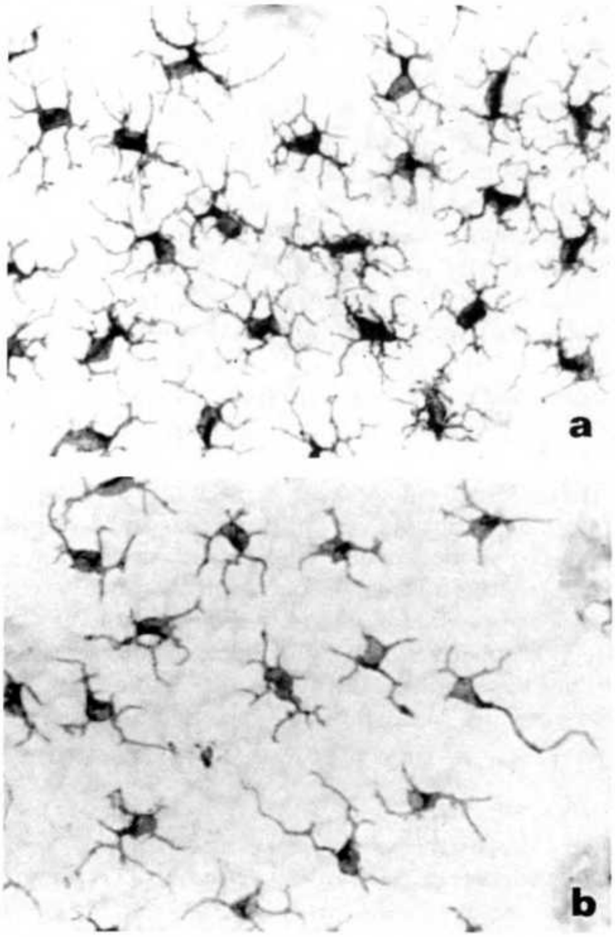

FIGURE 1. Epidermal sheet preparation showing Langerhans cells of mice on high-protein diet (a) and low-protein diet (b). Note the reduced density and decreased arborization in Langerhans cells from mice on a low-protein diet. Anti-Ia immunoperoxidase staining. (Original × 272).

TABLE 1. Langerhans cell density in epidermal sheets of mice on high and low-protein diet

	Langerhans cell number/mm^2*	
	ADPase staining	Ia immunoperoxidase
High-protein diet	806 ± 19	835 ± 26
Low-protein diet	412 ± 10	440 ± 13

* Mean ± SE.

Morphometric assessment

Langerhans cells of the low-protein group showed less dendrites than those of the control group (Fig. 1). To determine whether there had been a general atrophy of the Langerhans cell or whether there had only been a loss of arborization, morphometric assessment was performed. We found that the total cross-sectional area of Langerhans cell profiles in the low-protein group was significantly lower ($P < 0{\cdot}001$) than that of the control group (Table 2). The cross-sectional cell body area of the Langerhans cells from the low-protein diet group was also significantly smaller than that from the control group ($P < 0{\cdot}01$). Reduction of arborization accounted for most of the reduction of the total Langerhans cell cross-sectional area in the low-protein group (Table 2).

Quantitative cytophotometry

ADPase ectoenzyme activity and Ia antigen concentration were assessed quantitatively using a cytophotometer by measuring the mean absorbance values of the reaction products. Statistical data on the level of ADPase ectoenzyme activity and Ia antigen concentration in high and low-protein diets, as expressed in arbitrary units of absorbance, are presented in Table 3. Figures 2 and 3 illustrate the frequency distribution of the absorbance values by ADPase and Ia immunoperoxidase reaction products respectively, in high and low-protein diets.

The results showed that the activity of ADPase ectoenzyme in the low-protein diet group was reduced significantly ($P < 0{\cdot}001$). The mean absorbance value in the low-protein group was about half that in the control group. Langerhans cells' Ia antigen concentration was also significantly reduced after the low-protein diet ($P < 0{\cdot}001$).

TABLE 2. Langerhans cell morphometry of mice on high and low-protein diet

	Total cell area* (μm^2)	Cell body area* (μm^2)	Arborization area* (μm^2)
High-protein diet	162·55 ± 3·38	61 10 ± 0 86	101·45 ± 2·89
Low-protein diet	118 48 ± 2·22	57·70 ± 0·71	60·78 ± 1 91

* Mean ± SE.

TABLE 3. ADPase activity and Ia antigen concentration in Langerhans cells of high and low-protein diets expressed as mean absorbance values in arbitrary units

	ADPase*	Ia antigen*
High-protein diet	4658·63 ± 85 44	4494·03 ± 45·21
Low-protein diet	2319 03 ± 73·61	3822·35 ± 52·22

* Mean ± SE.

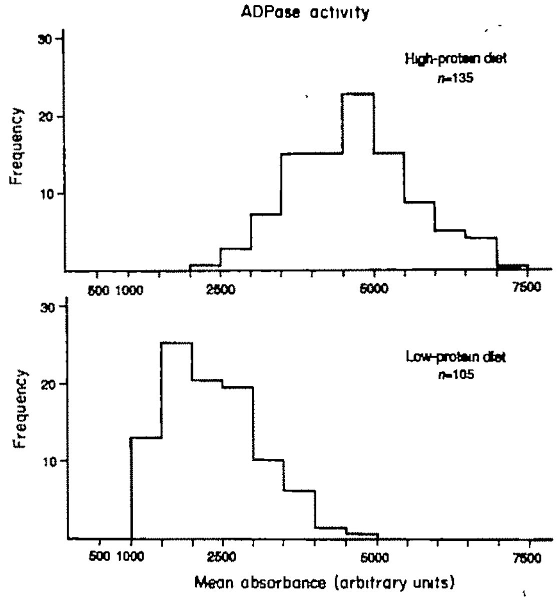

FIGURE 2. Comparison of ADPase ectoenzyme activity in high and low-protein diet as detected by cytophotometry. The concentration of membrane ADPase in Langerhans cells from mice on low-protein diet is decreased.

DISCUSSION

In these series of experiments we found that a low-protein diet induced reduction of Ia and ADPase positive LC density, atrophy of the cells with reduction of arborization, and decreased concentrations of ADPase ectoenzyme and Ia antigen.

It is not certain whether the reduction of Langerhans cell number as detected by ADPase and anti-Ia immunoperoxidase techniques reflects a true depletion of the cells, or merely represents a modulation of the expression of these markers. By employing two different and unrelated markers as a check on each other, the latter possibility was minimized. The density of LCs detected by both markers either in high or low-protein group (Table 1) is not significantly different ($P > 0{\cdot}05$). This suggests that the two markers, at least in the mouse system, identify the same cell populations and it is, therefore, more likely that the reduction observed in protein malnourished mice is a true depletion of Langerhans cells.

The mechanism by which Langerhans cell number is maintained is still uncertain. Studies using ^{3}H-thymidine incorporation revealed that murine Langerhans cells had a labelling index of less than 0·01%, which suggests that the turnover of Langerhans cells is very slow.[14] An occasional labelled Langerhans cell has also been observed in rhesus monkey.[15] Tamaki *et al.*[16] proposed that murine LCs are continually repopulated by precursor cells originating in the bone marrow. On the other hand, Czernielewski *et al.*,[17] using flow cytometric technique, reported that 1·3–3·3% of human Langerhans cells are in S phase and 1·0–2·5% in G_2/M, suggesting that LCs may represent a stable, self-reproducing cell population. These discrepancies may be due to a species difference.

The density of Langerhans cells has certain functional implications. A critical density of Langerhans cells is essential in the sensitization phase of contact hypersensitivity.[18,19] When

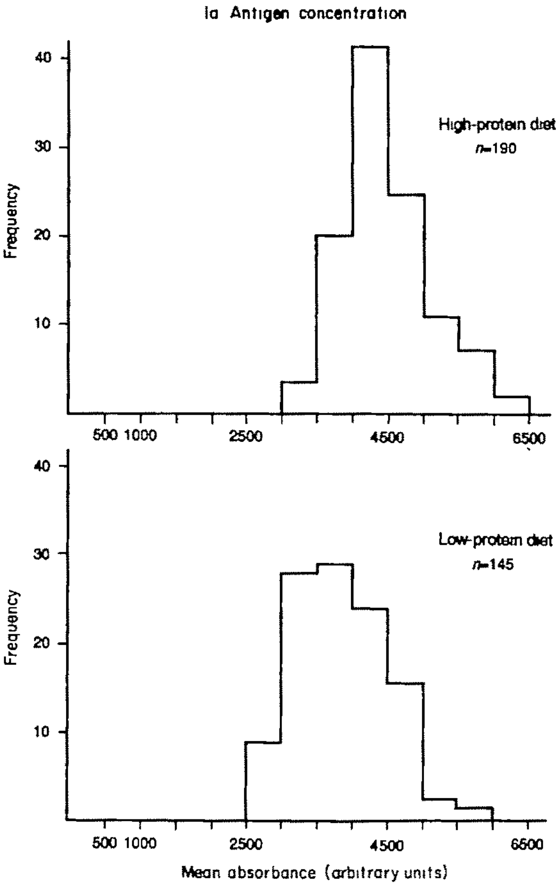

FIGURE 3. Comparison of Ia antigen concentration in high and low-protein diet measured by cytophotometry. Ia antigen concentration on LCs of mice on low-protein diet is reduced.

mice are initially exposed to dinitrofluorobenzene (DNFB) on skin naturally deficient in Langerhans cells (tail skin) or artificially depleted by UVB irradiation, they do not become sensitized. It has been concluded, therefore, that the number and density of LCs determine whether specific sensitization or unresponsiveness occurs.[18,19] A primary disturbance to DNCB hypersensitivity in protein-calorie malnutrition has been reported.[1,2] Reduction of Langerhans cell density could partially explain the failure of the afferent phase of cutaneous hypersensitivity in protein malnutrition.

Our findings, however, are not in agreement with the studies of Fox *et al.*[20] The latter found that in patients with malignancy there were no significant differences of Langerhans cell density with respect to nutritional status. Furthermore, they found no significant differences in the dendritic nature, fluorescent staining intensity, or size of HLA-DR antigen-bearing epidermal Langerhans cells within the epidermis of the patients studied. One possible reason stated for these discrepancies is that their patients were not severely nutritionally deprived.[20] In our study, variables such as age, body weight, duration and type of diet were standardized, facilitating reproducible and more reliable results.

Expression of Ia antigens is an absolute requirement for antigen presentation.[21,22] Any process that modulates their expression may significantly influence immune responses.[21] Unfortunately, quantitative data on the minimal Ia concentration needed for Langerhans cells to function properly are not yet available. The significance of the decrease of Ia antigen concentration on murine Langerhans cells after a low-protein diet in this experiment is still unclear.

It has been proposed that Langerhans cell dendrites form a network to trap antigens.[23] It is also possible, however, that arborization is the means whereby Langerhans cells present their maximal immunologically reactive surface area, and that reduction of arborization in protein malnutrition would decrease their functional capacity.

The exact function of nucleoside phosphatase on the cell membrane of the Langerhans cell is not yet known. Wolff[24] speculated that membrane ATPase is indicative of a transmembrane transfer mechanism that is important in the intercellular interaction between Langerhans cells and keratinocytes. Riley suggested that the function of Langerhans cells ATPase ectoenzyme is similar to that of actomyosin ATPase[25] and supports the notion that the Langerhans cell is a migratory cell.[25] It is interesting to note that the concentration of ADPase ectoenzyme in Langerhans cells after low-protein diet is decreased. Assuming that these nucleoside phosphatases are important in Langerhans cell motility,[25] defects of this function may occur in protein malnutrition.

In conclusion, this investigation has shown that a low-protein diet induces decreased Langerhans cell density, atrophy of cell bodies, loss of arborization, decreased ADPase ectoenzyme activity, and decreased Ia antigen concentration. We postulate that these changes would affect Langerhans cell function and eventually influence cutaneous immune responses. Further experiments are being conducted to test this hypothesis.

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REFERENCES

1 Gross RL, Newberne PM. Role of nutrition in immunologic function. *Physiol Rev* 1980; **60:** 188–302.

2 Edelman R. Cell mediated immune response in protein–calorie malnutrition—a review. In: *Malnutrition and the Immune Response* (Suskind RM, ed.). New York: Raven Press, 1977; 47–76.

3 Lamont AG, Gordon M, Ferguson A. T lymphocytes function in protein deprived mice. *Clin Exp Immunol* 1988, **72:** 113–7.

4 Passwell JH, Steward MW, Soothill JF. The effects of protein malnutrition on macrophage function and the amount and affinity of antibody response. *Clin Exp Immunol* 1974; **17:** 491–5.

5 Rose AH, Holt PG, Turner KJ. The effect of a low protein diet on the immunogenic activity of murine peritoneal macrophages. *Int Arch Allergy Appl Immunol* 1982; **67:** 356–61.

6 Stingl G, Katz SI, Shevach EM *et al.* Analogous functions of macrophages and Langerhans cells in the initiation of the immune response. *J Invest Dermatol* 1978, **71:** 59–64.

7 van Furth R. Mononuclear phagocyte of the skin. In: *Proceedings of the XVIth International Congress of Dermatology* (Kukita A, Seifi M, eds.). Tokyo: University of Tokyo Press, 1983; 47–58.

8 Thorbecke GJ, Silberberg-Sinakin I, Flotte TJ. Langerhans cells as macrophages in skin and lymphoid organs. *J Invest Dermatol* 1980; **75:** 32–43.

9 Bell RG, Hazell LA, Price P. Influence of dietary protein restriction on immune competence. II. Effect on lymphoid tissue. *Clin Exp Immunol* 1976; **26:** 314–26.

10 Mackenzie IC, Squier CA. Cytochemical identification of ATPase-positive Langerhans cells in EDTA-separated sheets of mouse epidermis. *Br J Dermatol* 1975; **92:** 523–33.

11 Chaker MB, Tharp MD, Bergstresser PR. Rodent epidermal Langerhans cells demonstrate greater histochemical specificity for ADP than for ATP and AMP. *J Invest Dermatol* 1984; **82**: 496–500.

12 Falini B, Taylor CR. New developments in immunoperoxidase techniques and their application. *Arch Pathol Lab Med* 1983; **107**: 105–17.

13 van der Ploeg M, van den Broek K, Smeulders AMW *et al.* HIDACSYS: computer programs for interactive scanning cytophotometry. *Histochem* 1977; **54**: 273–88.

14 Mackenzie IC. Labelling of murine epidermal Langerhans cells with ^{3}H-thymidine. *Am J Anat* 1975; **144**: 127–36.

15 Giacometti L, Montagna W. Langerhans cells. uptake of tritiated thymidine. *Science* 1967; **157**: 439–40.

16 Tamaki K, Stingl G, Katz SI. The origin of Langerhans cells. *J Invest Dermatol* 1980; **74**: 309–11.

17 Czernielewski J, Vaigot P, Prunieras M. Epidermal Langerhans cells—a cycling cell population. *J Invest Dermatol* 1985; **84**: 424–6.

18 Streilein JW, Toews GT, Gilliam JN, Bergstresser PR. Tolerance or hypersensitivity to 2,4-dinitro-1-fluorobenzene: the role of Langerhans cell density within epidermis. *J Invest Dermatol* 1980; **74**: 319–22.

19 Lynch DH, Gurish MF, Daynes RA. Relationship between epidermal Langerhans cell density ATPase activity and the induction of contact hypersensitivity. *J Immunol* 1981; **126**: 1892–7.

20 Fox JL, Berman B, Goodman R. Reduced epidermal Langerhans cell density in patients with pulmonary malignancy do not correlate with anergy or nutritional status. *J Dermatol Surg Oncol* 1985; **11**: 1087–95

21 Unanue ER, Beller DI, Lu CY, Allen PM. Antigen presentation: comments on its regulation and mechanism. *J Immunol* 1984; **132**: 1–5.

22 Braathen LR, Bjerke S, Thorsby E. The antigen-presenting function of human Langerhans cells. *Immunobiology* 1984; **168**: 301–12.

23 Shelley WB, Juhlin L. Langerhans cells form a reticuloepithelial trap for external contact allergens. *Nature* 1976; **261**: 46–7.

24 Wolff K, Winkelmann RK. Quantitative studies on the Langerhans cell population of guinea-pig epidermis. *J Invest Dermatol* 1967; **48**: 504–13.

25 Riley PA. A histochemical study of polyphosphate hydrolases in epidermal dendritic cells. *J Invest Dermatol* 1966; **47**: 412–20.

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Identification of the origin of cells in human basal cell carcinoma xenografts in mice using *in situ* hybridization

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SUMMARY

In an attempt to identify more clearly the origin of cells in human basal cell carcinoma xenografts in mice, paraffin and frozen sections were subjected to *in situ* DNA hybridization with biotin labelled human and murine DNA probes. Human skin and mouse skin sections were used as controls. As expected, the implanted epithelium reacted with the human DNA probe and the surface epithelium and most of the stromal cells reacted with the murine probe. However, the stroma immediately surrounding the implanted epithelium contained cells of human origin mixed with murine cells. Occasional murine cells (presumed inflammatory) were present in the human implanted epithelium. Assessment showed no correlation between the degree of differentiation of the implanted epithelium and the ratio of human/murine cells in the contiguous stroma. This technique provides a sensitive test for identifying human cells in xenografts and may be useful in assessing the role of stromal cells in the differentiation of a variety of carcinomas.

It is well known that an epithelial-stromal interaction is involved in the growth and development of normal epithelium. For example, it has been shown that the basal cells of epidermal explants from 11-day-old chick embryos lose their morphology and mitotic ability, but when dermis is added to these explants, these features are restored.[1] In adult epidermis, the epithelial–stromal interaction is equally important, as illustrated by Billingham and Silvers[2] who, using transplanted recombinant guinea-pig skin grafts, showed that the type of epidermis developing, depended on the type of dermis with which it had been combined.

Subsequently, it has been postulated that an abnormality in this epithelial stromal relationship may be involved to some degree in the genesis of at least some epithelial tumours. In the case of basal cell carcinomas, Pinkus[3] has suggested that the well defined distinctive

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connective tissue stroma, which can always be discerned on routine haematoxylin and eosin stained sections, is responsible for the neoplastic growth of the epithelial cells. Various studies have attempted to elicudate this relationship[4,5] but the results have been conflicting and difficult to interpret, largely for technical reasons.[6] We are currently investigating the role of the stromal regulation of growth of basal cell carcinoma epithelia.[6] This has involved transplanting basal cell carcinomas into the skin of nude mice, and examining the relationship between human and murine stroma and the growth and differentiation of the basal cell carcinoma epithelium. The experiments have yielded tumour nodules showing a full spectrum of epithelial differentiation (Fig. 1). Some of the epithelium consisted solely of basal cell carcinoma, some solely of keratinized squamous cell cysts and skin adenexae, and others of differentiated squamous cell cysts with plaques of basal cell carcinoma within their walls. However, attempts to determine the species origin of the surrounding stromal cells using existing techniques, i.e. human specific antibodies and Hoescht dye fluorescence, have been unsatisfactory.[6]

In an attempt to overcome this problem we have used *in situ* DNA hybridization to distinguish the species origin of the cells within the xenografts.

METHODS

Xenografts

The preparation, growth and harvesting of the basal cell carcinomas were performed as previously described.[6] Briefly, 37 human basal cell carcinomas were used. The delay between excision and grafting was less than 6 h. As much non-tumour tissue as possible was removed under a dissecting microscope. The tumour tissue was placed into fresh antibiotic supplemented tissue culture medium, and minced into fragments no larger than 1 mm. The fragments

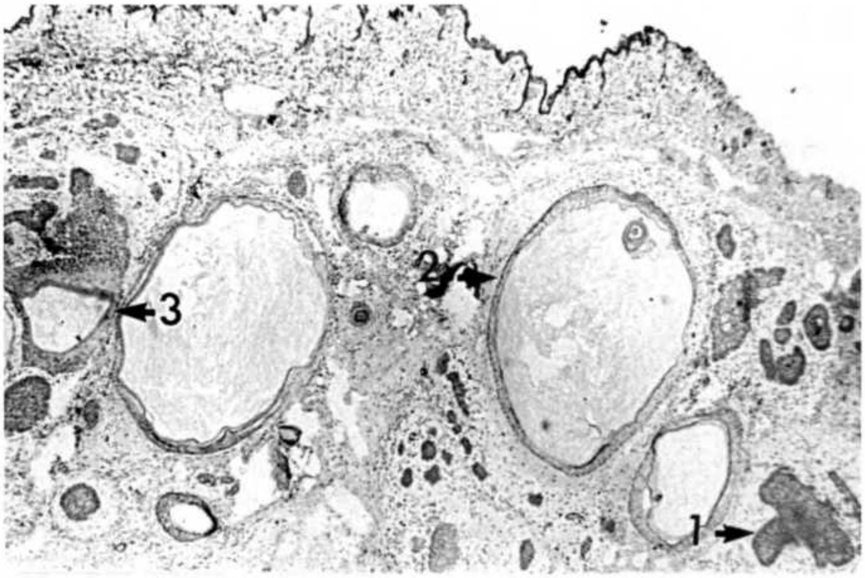

FIGURE 1. Human basal cell carcinoma xenograft in subcutaneous skin of a mouse. Top: Murine squamous cell epithelium. Below: Tumour nodule. Arrow 1: human basal cell carcinoma epithelium. Arrow 2: keratinized human squamous epithelium forming cysts. Arrow 3: squamous cell cysts with plaques of basal cell carcinoma within their walls.

were introduced into small subcutaneous pockets on the backs of NIMR athymic mice. Four grafts were lost, the remainder being removed between 38 and 150 days. Some were snap frozen, some paraformaldehyde fixed, and others formalin fixed and paraffin embedded.

Probes

The DNA from human white blood cells and murine liver cells was extracted by a standard phenol method.[7] The resultant high molecular weight DNA was nick translated and labelled with biotin (Bethesda Research Laboratories). The plasmid pBR 322 was used as a negative control.

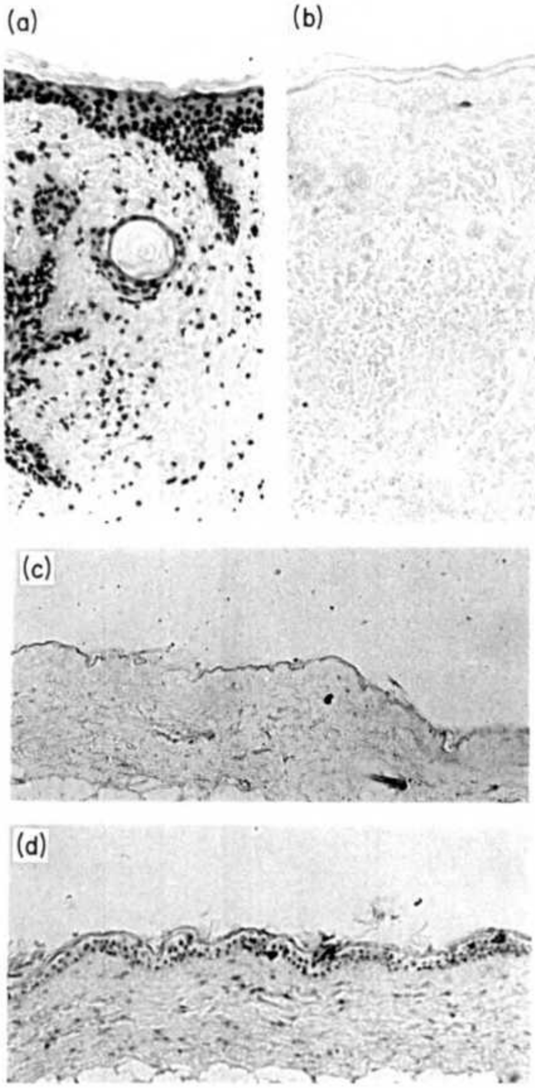

FIGURE 2. (a) Human skin with human DNA probe applied. (b) Human skin with murine DNA probe applied. (c) Murine skin with human DNA probe applied. (d) Murine skin with murine DNA probe applied.

Controls

Sections of human skin containing basal cell carcinoma which had been formalin fixed and paraffin embedded, were used as controls. The murine skin covering the xenografts served as an internal control for the DNA probes.

In situ *DNA hybridization*

This was performed as described previously.[8] Briefly, paraffin embedded sections were dewaxed with three 5-min treatments with xylene and three 5-min treatments with acetone. The sections were then digested in a solution of pepsin, 1 mg/ml in 200 mM hydrochloric acid, at

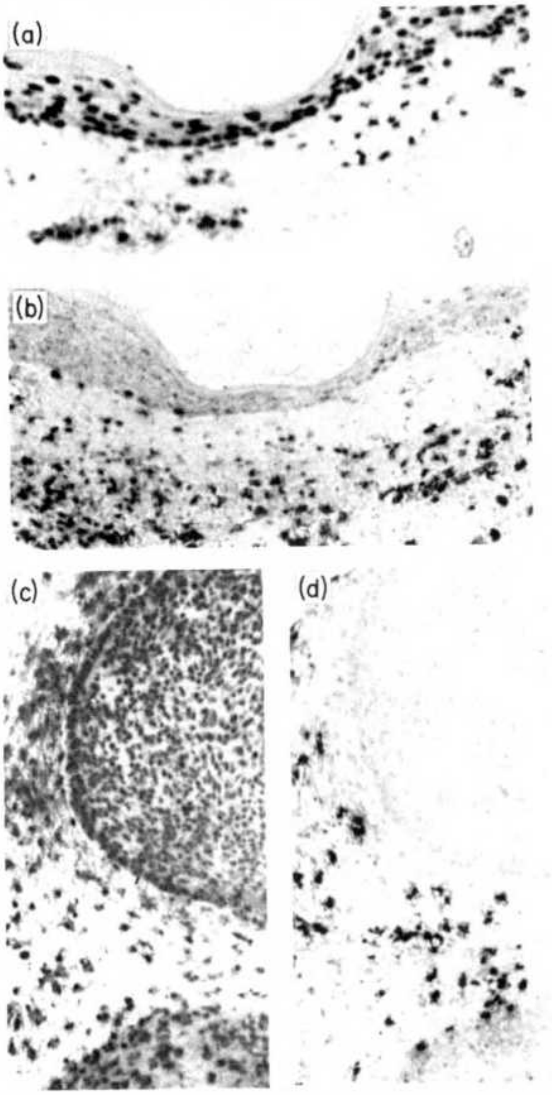

FIGURE 3. (a) Differentiated squamous cell cyst and surrounding stroma with human DNA probe applied. (b) Differentiated squamous cell cyst and surrounding stroma with murine DNA probe applied. (c) Non-keratinizing basal cell carcinoma and surrounding stroma with human DNA probe applied. (d) Non-keratinizing basal cell carcinoma and surrounding stroma with murine DNA probe applied.

37°C for 15 min, and rinsed with distilled water and allowed to air dry. The probes were applied to the sections at concentrations between 1 μg/ml and 150 ng/ml and denatured beneath a coverslip for 7–10 min at 90°C. Subsequently, hybridization was performed at 37°C for 3 h. The coverslips were then removed and the sections washed with 0·5% phosphate buffered saline (PBS). The sections were blocked with 5% bovine albumin solution for 20 min at 37°C. The slides were rinsed and incubated with a 1:250 dilution of avidin alkaline phosphatase conjugate (Dakopatts, Denmark) for 30 min at 37°C. The sections were washed with PBS with 0·5% Triton, then PBS followed by alkaline phosphatase incubation buffer, before being developed in nitro blue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate solution in the dark, at room temperature for 45 min. On 19 of the well stained nodules, the ratio of human to murine stromal cells was assessed by counting the number of human (H) to murine (M) cells within graticuled areas of 1 mm^2 at four points around the transplanted epithelium. The ratios were tabulated as M > H or M < H if more than two-thirds of the cells were of one type and as M = H if there was no such distinction.

RESULTS

The probes reacted in an appropriate manner with the tissue sections, i.e. the human probe reacted with nuclei in human skin and not with nuclei in murine skin and vice versa (Fig. 2). The results were equally reproducible in frozen sections or in paraformaldehyde or formalin-fixed paraffin-embedded tissues. However, morphology was best in formalin-fixed paraffin-embedded blocks. Of the 26 tumour nodules tested, four contained no human elements, three were poorly stained and 19 were well stained and showed clear differentiation between human and murine cells. The epithelia of the inclusion cysts and skin adenexae in the tumour nodules reacted with the human probe as did the non-keratinizing basal cell carcinoma epithelium (Fig. 3a and c). The stroma around the tumour nodules contained a mixture of cells which reacted with either the human or the murine probe (Fig. 3). The ratios of human to murine stromal cells around 13 foci of differentiated, stratified squamous cell cysts, 10 foci of basal cell carcinoma and three foci of mixed structures, i.e. differentiated cysts with plaques of basal cell carcinoma within their walls, are shown in Table 1. A depth of five cells from the adjacent epithelium was not exceeded. No distinction was made between fibroblasts, endothelial cells and inflammatory cells, and all were counted.

TABLE 1. Ratios of human (H): murine (M) stromal cells in 19 tumour nodules examined by *in situ* hybridization

Ratio of human: murine stromal cells*	No. of structures differentiated	BCC	Mixed
M=H	6	4	3
M>H	5	5	—
H>M	2	1	—
Total	13	10	3

*M=H: ratio of two cell types is less than 2:1.
M>H: ratio murine to human cells is greater than 2:1.
H>M: ratio of human to murine cells is greater than 2:1.

DISCUSSION

Basal cell carcinomas are characterized by the universal presence of a distinct stroma around the epithelial cells. It has been suggested that the growth and differentiation of basal cell carcinoma epithelium is dependent on this stroma.[3] Evidence in support of this theory has been presented by Scott,[9] who showed that basal cell carcinoma cells could be made to differentiate, i.e. keratinize, by autotransplanting them into new connective tissue beds. In addition Flaxman[5] has demonstrated that, *in vitro*, basal cell carcinoma cells grow as an epithelial sheet in which the centre is composed of stratified squamous epithelium. In young cultures, the edges of these sheets are composed of monolayers of undifferentiated basal cell carcinoma cells, while in older cultures they are composed of differentiated epithelial cells.

Pinkus[3] has shown that basal cell carcinomas (that resemble human tumours) induced in rats could be made to differentiate into keratinizing squamous epithelial cysts when transplanted into the uterus of oestradiol-treated virgin female rats. He proposed the hypothesis that the ability for neoplastic growth of the epithelial cells of basal cell carcinomas depends on its unique stroma. He conceded, however, that this may not be the only explanation, since he could not rule out other factors in the experiment, such as the specific influences in the oestradiol stimulated uterus.

In contrast to Pinkus' work, Grinwood[4] disputed the occurrence of this differentiation, since he showed that transplanted human basal cell carcinomas in immuno-suppressed athymic nude mice, whilst continuing to grow, did not become more undifferentiated, or mature in a manner different from the original tumour. He failed, however, to account for the epithelial inclusion cysts seen in some of his nodules, a finding present in our model system. While we cannot exclude the possibility that these differentiated structures represent included normal human epithelial cells, it is equally possible that they represent differentiated basal cell carcinoma cells, now no longer under the influence of their unique stroma.

In our experimental model, if Pinkus' theory is correct, then the stroma around the undifferentiated epithelium would be expected to be predominantly the unique human stroma and thus stain predominantly with the human probe. The differentiated structures, on the other hand, would be expected to be surrounded by a stroma lacking or showing a decrease in the number of human cells. Our results show that, whilst the differentiated structures, in general, showed a predominance of murine cells (in 11 of 13, M:H ratio > 2:1) consistent with Pinkus' theory, the undifferentiated basal cell epithelium also showed a similar predominance (in 9 of 10, M:H ratio > 2:1). These results indicate that the undifferentiated neoplastic growth of basal cell carcinoma cells is not solely dependent on its associated unique stroma and that other factors must be of relevance.

These experiments also illustrate the usefulness of *in situ* hybridization in identifying the species of origin of cells xenografts. *In situ* DNA hybridization using biotinylated probes is a quick, easy and reliable method which offers a clear comparison with haematoxylin and eosin stained sections. It has the additional advantage over radioactive probes, as used previously,[10] of cheapness, high resolution, safety and probe stability.

REFERENCES

1 Jarret A. *The Physiology and Pathophysiology of The Skin, Vol 1. The Epidermis*. London: Academic Press, 1973.
2 Billingham RE, Silvers WK. The origin and conservation of epidermal specificities. *New Engl J Med* 1963; **268:** 477–80, 539–45.

3 Pinkus H, Cooper M. Intrauterine transplantation of rat basal cell carcinoma as a model for reconversion of malignant to benign growth. *Cancer Res* 1977; **37:** 2544–52.

4 Grinwood RE, Johnson CA, Ferris CF *et al.* Transplantation of human basal cell carcinomas to athymic mice. *Cancer* 1985; **56:** 519–23.

5 Flaxman BA. Growth in vitro and induction of differentiation of basal cell carcinomas. *Cancer Res* 1972; **32:** 462–9.

6 Stamp G, Braithwaite A, Quaba A. Basal cell carcinoma xenografts in athymic mice. *J Pathol* 1988; **154:** 45A.

7 Biochemical Techniques (Appendix A). In: *Molecular Cloning. A Laboratory Manual* (Maniatis T, Fritsch EF, Sambrook J, eds). New York: Cold Spring Harbor Laboratory; 1982; 458–9.

8 Porter HJ, Khong TY, Evans MF. *et al* Parvovirus as a cause of hydrops fetalis; detection by in situ DNA hybridization. *J Clin Pathol* 1988; **41:** 381–3.

9 Scott V. The modulating influence of stromal environment in epithelial cells studied in human autotransplants. *J Invest Dermatol* 1961; **36:** 109–31.

10 Obara T, Conti CJ, Baba M *et al* Rapid detection of xenotransplanted human tissues using *in situ* hybridization. *Am J Pathol* 1986; **122:** 386–91.

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Infantile seborrhoeic dermatitis and *Pityrosporum ovale*

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SUMMARY

Twenty children (mean age 9 weeks) with infantile seborrhoeic dermatitis (ISD) were investigated with cultures for bacteria, *Pityrosporum ovale* and other fungi, and 20 healthy children served as controls. *P. ovale* and *Staphylococcus aureus* were the dominant organisms. *P. ovale* was cultured in 18 of 20 infants with ISD as compared to 4 of 20 controls. *S. aureus* was cultured in 14 of 20 infants with ISD as compared to 1 of 20 controls. The role of *S. aureus* in ISD is not known, but it could be a secondary invader as is supposed in atopic dermatitis (AD). Even if *P. ovale* may be suspected as the aetiological agent of ISD further studies are needed to clarify the exact role of the organism in ISD.

Infantile seborrhoeic dermatitis (ISD) is considered by some to be a distinct entity, while others think that it is a clinical variant of atopic dermatitis (AD).[1] ISD has been compared with the post-pubertal seborrhoeic dermatitis, but its relationship to adult disease is obscure. *P. ovale* is recognized as a saprophytic organism found in all normal adults but it is uncommon in healthy children.[2–4] *P. ovale* is also responsible for different kinds of skin diseases, such as pityriasis versicolor,[2] seborrhoeic dermatitis in adults,[5] Pityrosporum folliculitis[2] and confluent and reticulate papillomatosis (Gougerot–Carteaud).[2,4] The pathomechanisms of ISD are unknown although bacterial and yeast infections have been suggested. An allergic reaction to Candidal antigens is one of the hypotheses.[6] The relation between ISD and *P. ovale* has not been studied. The aim of the present study is to culture bacteria and fungi including *P. ovale* from patients with ISD.

METHODS

Patients

Twenty infants, nine females and 11 males, with a mean age of 9 weeks (range 4–16 weeks) were included. They had a clinical picture of ISD with erythematous squamous scaly eczema involving scalp, face and napkin area.[7] The mean age at onset was 4 weeks (range 1 week–12 weeks). The parents were asked about the family history concerning atopic disease and other skin disorders. Twenty healthy children, eight females and 12 males, with a mean age of 7 weeks (range 3 weeks–12 weeks) served as controls.

Experimental design

In all patients and controls the following studies and investigations were done: direct microscopy for fungi, qualitative and quantitative culture for *P. ovale* and qualitative culture for other fungi and bacteria. All samples were taken from the forehead or temporal area. Specimens for direct microscopy were obtained by cellophane tape, stained with methylene blue and mounted on a microslide. Qualitative cultures for *P. ovale* were taken with a curette and transferred to a glucose neopeptone yeast extract medium with lipid supplements previously described.[2] Quantitative culture for *P. ovale* was obtained by the use of contact plates (PDM Pityrospoum Contact Plates AB, Biodisk, Solna, Sweden).[8] Briefly, the contact plate was pressed against the skin area for 15 s, incubated in a Bio-Bag SFJ (Marion Laboratories, Kansas City, U.S.A.) at 37°C and read after 6 days. Specimens for culture of other fungi were transferred to Sabouraud's agar without supplements and incubated at 37°C for up to 1 week. Material for bacterial culture was taken with a cool impregnated cotton swab, transferred to TMG-transport medium and sent to the microbiological laboratory.

TABLE 1. Results of cultures from lesional skin in 20 children with ISD

	P. ovale microscopy	Qual culture	Quant. culture	Other fungi cultures	Bacterial cultures
1	few	+*	0	*C. albicans*	Other†
2	many	+	10	neg	*S. aureus*
3	few	+	1	neg	*S. aureus* Other
4	few	+ + +	11	*C. albicans*	*S. aureus*
5	neg	+ +	23	neg	*S. aureus* Other
6	many	+ +	4	neg	Other
7	few	+ +	24	neg	*S. aureus*
8	few	+ +	8	neg	*S. aureus*
9	few	+	6	neg	*S. aureus*
10	few	+	1	neg	Other
11	neg	+ +	8	neg	Other
12	few	+ +	47	neg	*S. aureus*
13	few	+ +	12	neg	*S. aureus* *St. hemolyt. gr. B*
14	many	+ + +	0	neg	Other
15	many	+ +	0	neg	Other
16	few	+	16	neg	*S. aureus*
17	few	+	9	neg	*S. aureus*
18	few	0	0	neg	*S. aureus*
19	neg	0	0	neg	*S. aureus*
20	neg	+	2	neg	*S. aureus*

* + = < 5 col, + + = 5–10 col, + + + = > 10 col.

† Other included = *S. epidermidis*, α-Streptococcus, *St.gr.B.*

Statistics

The Chi-square test with Yates' correction was used to compare the number of positive fungal and bacterial cultures in the two groups.

RESULTS

In 20 patients with ISD, a family history of AD was seen in eight children, and in the control group in 10 children. Other skin disorders were seen in two children in the ISD group (two cases of adult seborrhoeic dermatitis), and in four children in the control group (one case of contact eczema, one case of psoriasis, one case of eczema, and one case of cold urticaria). The area of onset was the scalp in six cases, the face in 12 cases, the napkin area in one case and the neck region in one case.

The results of the culture studies in the children with ISD are shown in Table 1. Table 2 shows the results of the culture studies of the 20 control children.

P. ovale and *S. aureus* were the dominant organisms cultured in the children with ISD. Both of these organisms were cultured in significantly higher numbers of children with ISD compared to controls $(P < 0{\cdot}01)$.

TABLE 2. Results of skin cultures in 20 healthy control children

	P. ovale microscopy	Qual. culture	Quant. culture	Other fungi cultures	Bacterial cultures
1	neg	+*	8	neg	Other†
2	neg	0	0	neg	neg
3	many	+	1	neg	Other
4	few	+	3	neg	neg
5	neg	0	0	neg	neg
6	neg	0	0	neg	Other
7	neg	0	0	neg	Other
8	neg	0	0	neg	neg
9	neg	0	0	neg	neg
10	neg	0	0	neg	neg
11	neg	0	0	neg	Other
12	neg	0	0	neg	neg
13	neg	0	0	neg	neg
14	few	+	0	neg	*S. aureus*
15	neg	0	0	neg	neg
16	neg	0	0	neg	neg
17	few	0	0	neg	neg
18	neg	0	0	neg	neg
19	few	0	0	neg	Other
20	neg	0	0	neg	Other

* + = < 5 col, + + = 5–10 col, + + + = > 10 col.

† Other included = *S. epidermis*, α-streptococcus, *St. gr. B.*

DISCUSSION

ISD is a skin disorder in infants usually starting before the age of 4 months. The skin lesions are red and scaly often starting in the scalp and involving the flexures or the napkin area.[9] The child is usually unaffected by the disease even when it is widespread. There are no generally accepted diagnostic criteria for ISD, unlike AD. The criteria in AD as stated by Hanifin and Rajka[10] are difficult to apply in early infancy. ISD may be a distinct entity or a variant of AD.

The aetiology of ISD is unclear but *Candida albicans* has been mentioned as an important factor.[6] In the present study we could not find *C. albicans* in 18 of our patients. We noted a high frequency of colonization by *S. aureus* in the infants with ISD and in two of the children a clinical picture of bacterial infected eczema was noted. The role of *S. aureus* in ISD is not known but it could be a secondary invader as is supposed in AD. In one child *Streptococcus hemolyticus gr.B* was cultured from the eczema of the face and perianal region, and the mother had tonsillitis caused by the same organism.

P. ovale was cultured in 18 out of 20 infants with ISD as compared to four out of 20 healthy controls. The newborn display endocrine behaviour not unlike that of puberty[11] which could favour colonization with the lipophilic *P. ovale* but this does not explain the difference in the two groups. The number of days spent in an incubator is a risk factor and is thought to correlate with colonization with *P. ovale*.[4] This was not confirmed in our group of children. If, as we suspect, *P. ovale* is the aetiological agent of ISD, further studies are needed to clarify its exact role. The fact that postpubertal seborrhoeic dermatitis is a fungal disease is now well established.[9] The rapid response to topical (or systemic) Pityrosporicidal therapy is a diagnostic feature of postpubertal seborrhoeic dermatitis.[9,12] Studies in ISD on the effect of antifungal agents on the clinical condition could help to answer the question of whether *P. ovale* is the aetiological agent of ISD. Before using topical antifungals in ISD, further investigations as to their safety in children are necessary.

REFERENCES

1 Podmore P, Burrows D, Eedy DJ, Stanford CF. Seborrhoeic eczema—a disease entity or a clinical variant of atopic eczema. *Br J Dermatol* 1986; **115**: 341–50.

2 Faergemann J Lipophilic yeasts in skin disease. *Semin Dermatol* 1985; **4**: 173–84.

3 Faergemann J, Fredriksson T. Age incidence of Pityrosporum orbiculare on human skin. *Acta Derm Venereol (Stockh)* 1980; **60**, 531–3.

4 Powell D, Hayes J, Durell D *et al.* Malassezia furfur skin colonization of infants hospitalized in intensive care units. *J Pediatr* 1987; **111**: 217–20.

5 Shuster S. The aetiology of dandruff and the mode of action of therapeutic agents. *Br J Dermatol* 1984; **111**: 235–42.

6 Seebacher C. Zur Ätiologie und Pathogenese der Dermatitis seborrhoides infantum. *Mykosen* 1981; **24**: 209–15.

7 Atherton DJ, Rook A. Infantile seborrhoeic dermatitis. In: *Textbook of Dermatology* (eds Rook A, Wilkinson DS, Ebling FJG *et al.*), 4th ed. Oxford. Blackwell Scientific Publications, 1986; pp. 246–9.

8 Faergemann J. The use of contact plates for quantitative culture of *Pityrosporum orbiculare*. *Mykosen* 1987; **30**: 298–304.

9 Ive FA, Rowland Payne CME. Seborrhoeic dermatitis. In: *New Clinical Applications Dermatology—Superficial fungal infections* (Verbov JL ed.), Boston: MTM Press Limited, 1986, pp. 73–86.

10 Hanifin JM, Rajka G. Diagnostic features of atopic dermatitis. *Acta Derm Venereol (Stockh)*, 1980; Suppl **92**: 44–7.

11 Winther JSD, Faiman C, Hobson WC *et al.* Pituitary-gonadal relations in infancy. 1. Patterns of serum gonadotropin concentrations from birth to four years of age in man and chimpanzee. *J Clin Endocrinol Metab* 1975; **40**: 545–51.

12 Faergemann J. Seborrhoeic dermatitis and *Pityrosporum orbiculare*: treatment of seborrhoeic dermatitis of the scalp with miconazole-hydrocortisone. *Br J Dermatol* 1986; **114**: 695–700.

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Relationship of the final α-keratin compositions in dyskeratoses to those along the normal keratinization pathway: aetiological and diagnostic significance

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SUMMARY

The final α-keratin compositions attained in the outer horny layer of 16 dyskeratoses have been compared with the series of compositions which is produced during normal epidermal differentiation. In each case, the abnormal outer horny layer composition corresponded with that of normal basal, spinous, granular or inner horny cells, with, in some cases, the addition of α-keratins characteristic of hyperproliferating/regenerating keratinocytes. The results have implications for the aetiology of these diseases. In addition, the distinction between the α-keratin patterns in certain of the conditions was sufficiently clear to allow the use of the technique as a non-invasive aid to diagnosis.

During normal keratinization, human epidermis synthesizes, modifies and degrades several quantitatively major α-keratins. The sequence in which these events take place has been established by the combination of a variety of techniques.[1-3] These studies have demonstrated that human basal cells contain two major α-keratins, M_r 58 and 50 kD (Moll catalogue numbers 5 and 14).[4] Immediately suprabasally, spinous cells synthesize two further α-keratins M_r 70 and 56·5 kD (catalogue numbers 1 and 10) and the 58 and 50 kD chains are removed during the spinous and granular stages. In the granular and inner horny layers the 70 and 56·5 kD chains are processed into products of 65 and 55 kD, respectively. These events generate a continuously changing pattern of α-keratin composition which can be used as a sensitive marker for the stage of differentiation of keratinocytes.

It is now well established that in a number of dyskeratoses the epidermis produces a horny layer with an abnormal α-keratin polypeptide chain composition[5-7] demonstrating that changes have occurred to the above sequence of events. The abnormal compositions have been described

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after separation of the chains by SDS-PAGE, but only a few attempts have been made to correlate the changes seen with other factors in the disease processes such as hyperproliferation[7,8] or histological degree of keratinization.[7] No clear conclusion has so far emerged as to whether the α-keratin abnormalities are an important early feature in the aetiology of these conditions or are secondary to some other known defect.

The major possibilities for the production of such abnormal α-keratin compositions are: (1) synthesis of α-keratins or combinations of α-keratins which are not found in normal tissue; (2) abnormal post-synthetic modification of normally synthesized proteins; (3) failure to complete an otherwise normal programme of synthesis and modification. Possibilities (1) or (2) would produce compositions not found in normal tissue, whereas possibility (3) would lead to α-keratin patterns which are characteristic of earlier stages in normal differentiation being retained into the outer horny layer. Each mechanism has different implications for the possible role of α-keratin abnormalities in the pathogenesis of the dyskeratoses.

An opportunity to distinguish between the above possibilities has been provided by the direct demonstration of the α-keratin compositions of basal, spinous, granular and inner horny keratinocytes from normal human epidermis.[3] The present study was, therefore, undertaken in order to determine whether the α-keratin compositions of the outer horny layers of 16 dyskeratoses lie along this normal keratinization pathway. In addition, the results of the survey were assessed for the possible usefulness of α-keratin patterns as a diagnostic tool.

METHODS

Samples of outer horny layer were obtained from normal and diseased epidermis by scraping with a scalpel blade. After extraction for 10 min in boiling sample buffer (2% SDS/5% β-mercaptoethanol/10% glycerol in 50 mM Tris-HCl, pH 6·8) α-keratins were separated on 10% acrylamide gels by the technique of Laemmli[9] and visualized by staining with Coomassie blue.

During the course of various studies on psoriasis[5,8,10,11] more than 200 samples from chronic plaques obtained as described in the Discussion have been examined. Samples from other diseases were obtained from the most freely scaling areas. The numbers of samples in the present study were: normal epidermis (10), atopic dermatitis (30), pityriasis rubra pilaris (14), X-linked ichthyosis (13), palmo-plantar pustulosis (12), acquired ichthyosis (4), seborrhoeic dermatitis (4), ichthyosiform erythroderma (3), Netherton's syndrome (3), autosomal dominant ichthyosis (2), collodion baby (2), bullous ichthyosiform erythroderma (2), harlequin foetus (1), erythrokeratoderma variabilis (1), erythrokeratoderma progressiva (1) and Unna-Thost keratoderma (1).

RESULTS

Samples of outer horny layer were obtained from lesions on various non-callused sites of the trunk and limbs or from the forearms of normal individuals. The content of quantitatively major α-keratin polypeptide chains does not vary significantly between these sites, whereas such variations have been described in the minor chains;[12] therefore, only the major chains are described below.

Normal epidermis

In the outer horny layer of each normal individual studied, the 50 kD chain had been removed

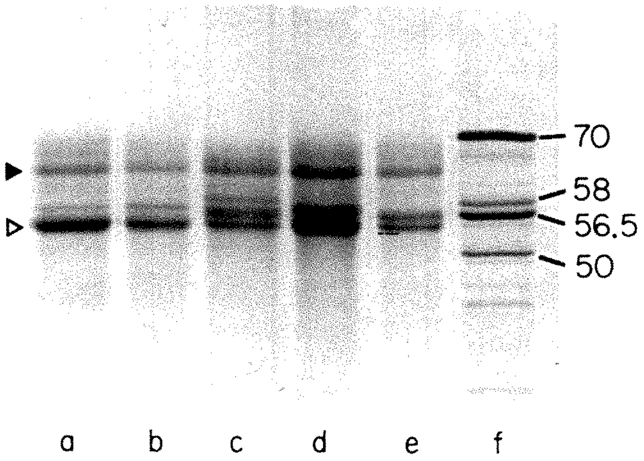

FIGURE 1. α-Keratin compositions of the outer horny layers of five normal individuals (tracks a–e) chosen to show the range of variation found. Track f is an extract of normal living cells showing the four major α-keratins M_r 70, 58, 56·5 and 50 kD. In each of the outer horny layer samples the 50 kD chain has been removed and its partner 58 kD chain was also removed in all but one (track c) of the total of 10 individuals studied. Processing of the 70 kD chain has, in each case, given rise to a major product of 65 kD (▶) with no detectable residual 70 kD and differing amounts of intermediates being present. The 56·5 kD chain has been processed to varying degrees into a product of 55 kD (▷). Thus, in tracks a and b the 55 kD chain is the major component of this pair, in track c and 56·5 kD chain is predominant and in tracks d and e the 55 and 56·5 kD chains are present in approximately equal amounts.

and the 58kD chain was undetectable in nine out of 10 cases. The 70 kD chain was absent so that the major chain of highest M_r was 65 kD. Between these there were bands of variable intensity representing intermediates in the processing of the 70 kD into the 65 kD chain.[1,13] The 56·5 kD chain content was also decreased in varying degrees by processing into a product of 55 kD. In no case were significant amounts of chains of <55 kD visible.

The data presented (Fig. 1) demonstrate that, in normal individuals, processing of the α-keratin chains occurs to varying degrees. The 65 kD processed product of the 70 kD chain is always the major component of that group of chains whereas the 55 kD product may be present in either major or minor amounts relative to its precursor 56·5 kD chain. Individuals with almost completely processed 56·5 kD chains can contain relatively unprocessed 70 kD chain intermediates and vice versa, showing that the processing of the two chains is not co-ordinated. Repeat samples taken from the same individuals 3 months later showed identical characteristics in terms of the removal and degree of processing of the major α-keratins, suggesting that the different patterns obtained were due to individual variation rather than fluctuation with time. The α-keratin compositions of the outer horny layers of the five normal individuals chosen to illustrate the range of variation found are shown in Figure 1.

Abnormal epidermis

The α-keratin composition of the outer horny layers of the skin diseases studied could be classified into four major types which are illustrated in Figure 2. Unless otherwise stated, the reported compositions were found in all examples of a particular disease type examined. They are grouped according to their content of major α-keratin chains and their similarity to compositions observed along the normal keratinization pathway.

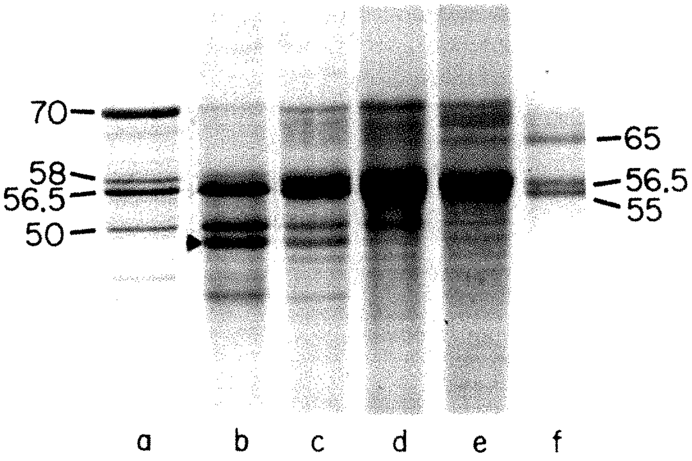

FIGURE 2. α-Keratin compositions from the four abnormal groups identified (tracks b–e) compared with extracts of normal living epidermal cells (track a—major α-keratins 70, 58, 56·5 and 50 kD) and normal outer horny layer (track f—major α-keratins 65, 56·5 and 55 kD). Track b is of a sample from a patient with psoriasis (Group 4); track c—pityriasis rubra pilaris (Group 3); track d—collodion baby (Group 2); track e—autosomal dominant ichthyosis (Group 1).

From left to right the conditions show increased production and processing of the 70 kD chain, increased processing of the 56·5 kD chain, increased removal of the 50 kD chain and production of the 48 kD chain (▶) in Groups 3 and 4. Group 1 conditions can, however, still be distinguished from normal (cf. tracks e and f) by the residual 70 kD chain and relative lack of 65 kD chain. The gel system was chosen particularly to resolve the 70 kD chain and its products. The distinction between group 1 and other conditions is more obvious if a system is used in which the 70 kD chain and its products run as one band.[8]

Group 1. In autosomal dominant ichthyosis, X-linked ichthyosis, acquired ichthyosis, ichthyosiform erythroderma and erythrokeratoderma variabilis, the 50 kD and 58 kD chains have been removed and the 56·5 kD chain partially processed to 55 kD. Processing of the 70 kD chain is, however, less complete than in normal outer horny layer, with there always being some residual 70 kD chain. This is a consistent difference from normal in which this chain is completely removed (cf. Figs 1 and 2) and the remaining 70 kD chain and its processing intermediates cause Group 1 conditions to resemble normal inner[2] rather than outer horny layer in their overall composition.

Group 2. In the harlequin foetus and collodion baby the 50 kD chain is not completely removed and the 70 kD chain is less processed than in the above group of conditions. This composition closely resembles that of normal granular cells.[2]

Group 3. In atopic dermatitis, seborrhoeic dermatitis, five out of 12 cases of palmo-plantar pustulosis, pityriasis rubra pilaris, Netherton's syndrome, bullous ichthyosiform erythroderma, Unna-Thost keratoderma and erythrokeratoderma progressiva, the 50 and 58 kD chains persist into the outer horny layer and the 70 kD and 56·5 kD chains are largely unprocessed. This composition is typical of normal spinous cells.[2] In addition, the epidermis in these diseases expresses a 48 kD α-keratin which is characteristic of hyperproliferating/regenerating keratinocytes.[7,14] The 56 kD chain which is paired with the 48 kD chain[7] would also be expected to be present, but is not resolved from the 56·5 kD chain by one-dimensional SDS-PAGE.

Group 4. In psoriasis and seven out of 12 cases of palmo-plantar pustulosis, the 50 kD and 58 kD chains persist into the horny layer and the 70 kD chain is either absent or greatly reduced in relative amount. α-Keratins of 48 kD and 56 kD are also present so that the overall α-keratin composition of the outer horny cells in these diseases resembles that of normal basal cells (50 kD and 58 kD chains) with the addition of the two chains (48 kD and 56 kD) which are characteristic of hyperproliferating/regenerating keratinocytes.

DISCUSSION

In the present study samples were obtained from a single stage of differentiation i.e. the outer horny layer. This has the advantages of: (1) being non-invasive; (2) avoiding the non-comparable mixtures of α-keratins from different stages of keratinization obtained when whole living layer or living plus horny layer cells are extracted; (3) obtaining α-keratins which have undergone all of the modifications to which they are subject in a particular condition. Some of the results are from conditions of which only a few samples were available. These cannot be regarded as necessarily typical of the diseases until further data are obtained. However they are included as they all fit the general pattern of being abnormal for outer horny layer, but fall along the normal differentiation pathway.

Of the conditions for which a greater number of samples was obtained only palmo-plantar pustulosis was clearly divisible into two groups with one group having a Group 4 α-keratin composition similar to that of psoriasis and the other a Group 3 composition. It is not known from clinical or other data whether this disease is a separate entity or a variant of psoriasis. Individuals in the two groups defined on the basis of α-keratin composition did not have any other mutually exclusive feature which might distinguish between these possibilities. Both groups consisted of females in the age range 50–65 years who showed no inter-group differences in their personal or family histories of psoriasis or in their response to treatment.

The results overall demonstrate that α-keratin composition is a sensitive indicator of abnormal differentiation in benign scaling disorders, each of which showed some difference from normal in the major polypeptides present in the outer horny layer. We have extended previous studies by showing that all of the compositions obtained represent stages along the normal differentiation pathway with, in some cases, the addition of two α-keratins produced by hyperproliferating/regenerating keratinocytes. These additional α-keratins (48 kD and 56 kD) have previously been reported to be produced in disease states by living keratinocytes[7] and have here been shown to persist into the outer horny layer. As they are also produced by normal epidermis induced to hyperproliferate[7,8,14] it can be concluded that the major α-keratins in the diseased outer horny layers studied exhibit no differences in chains or combinations of chains from those which can be produced by normal epidermis during normal or accelerated keratinization.

As the normal programme of synthesis, modification and degradation of the α-keratins does not take place, keratinization in these disorders can be considered to be incomplete in this respect. Furthermore, the spinous, granular or inner horny layer compositions seen in most of the conditions (Groups 1, 2 and 3) are produced in the outer horny layer of normal epidermis following tape stripping;[8] a treatment known to cause the epidermis to hyperproliferate. The retention of these compositions in skin diseases is, therefore, consistent with its being a consequence of epidermal hyperproliferation. Due to the current lack of sufficiently detailed and comparable kinetic data on most of the dyskeratoses studied here, it is not yet possible to make precise correlations between the α-keratin compositions which we have defined, and specific alterations in the patterns of cell division and migration.

The lack of normal expression of the 70 kD α-keratin in psoriasis however, is not immediately explicable in the above terms. There is no direct evidence that this phenomenon is a consequence of epidermal hyperproliferation whereas several lines of reasoning suggest that it is not. Firstly, in contrast to the other major α-keratins discussed above, the content of this protein in normal epidermis is not affected during experimentally-induced hyperproliferation.[8] Secondly, the 70 kD α-keratin is produced immediately suprabasally in normal epidermis,[15] but is not expressed in psoriatic epidermis despite the cells leaving the basal layer and having sufficient time to express other suprabasal characteristics. Thirdly, the patterns of expression of a variety of other markers of epidermal keratinization in psoriasis are consistent with a pathway of differentiation which is altered qualitatively rather than being simply incomplete as a consequence of hyperproliferation.[16,17]

As synthesis of the 70 kD/56·5 kD chain pair denotes the commitment of epithelial cells to keratinization with the loss of their proliferative ability[18] psoriasis is the only dyskeratosis amongst those studied in which such a commitment has not been made. Lack of expression of the 70 kD chain therefore appears to represent an early event in the pathogenesis of psoriasis, and is a readily detectable marker of the defective differentiation. Studies of factors influencing expression of this protein would, then, be of importance in understanding and influencing the development of psoriasis.

The classification of dyskeratoses according to their content of major α-keratin chains provides the opportunity for distinguishing between conditions in different groups using superficial scrapings and the relatively simple technique of one-dimensional SDS-PAGE. As the system is non-invasive and gives rapid results, it is potentially attractive as an aid to diagnosis. It must be stressed, however, that for effective use of the technique as a diagnostic tool it is essential to exercise close control over the sampling strategy, optimize the gel system used and establish the constancy of the α-keratin pattern derived from a substantial number of confirmed cases of the diseases under investigation. For example, in the course of studies on chronic plaque psoriasis we have sampled only stable or developing lesions several centimetres in diameter from the region midway between their centre and periphery. This avoids the areas and types of lesions most likely to go into remission with a concomitant increase in 70 kD chain and gives consistently low values for the 70 kD chain content.[5,8,10,11] We have further found that differences between diseases based on the amount of 70 kD chain plus its processed products are more pronounced than those based on the amount of 70 kD chain alone. For diagnostic use, this comparison is facilitated by the choice of a gel system (e.g. 12·5% acrylamide gels), in which these components run as a single band.

Using such procedures we have found that, in the diseases with which we have had the most experience, the α-keratin contents are remarkably consistent. As a result, it has proved possible to distinguish between psoriasis and pityriasis rubra pilaris or psoriasis and eczema in clinically equivocal cases, largely on the basis of the content of the 70 kD chain plus its products. Other distinctions should prove to be feasible as larger numbers of samples from other diseases are analysed.

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REFERENCES

1 Fuchs E, Green H. Changes in keratin gene expression during terminal differentiation of the keratinocyte. *Cell* 1980; **19:** 1033–42.

2 Skerrow D, Skerrow CJ. Tonofilament differentiation in human epidermis: Isolation and polypeptide chain composition of keratinocyte subpopulations. *Exp Cell Res* 1983; **143:** 27–35.

3 Woodcock-Mitchell J, Eichner R, Nelson WG *et al.* Immunolocalization of keratin polypeptides in human epidermis using monoclonal antibodies. *J Cell Biol* 1982; **95:** 580–8.

4 Moll R, Franke WW, Schiller DL *et al.* The catalog of human cytokeratins: patterns of expression in normal epithelia, tumours and cultured cells. *Cell* 1982; **31:** 11–24.

5 Skerrow D, Hunter I. Protein modification during the keratinization of normal and psoriatic human epidermis. *Biochim Biophys Acta* 1978; **537:** 474–84.

6 Baden HP, Lee LD. Fibrous proteins of human epidermis. *J Invest Dermatol* 1978; **71:** 148–51.

7 Weiss RA, Eichner R, Sun T-T. Monoclonal antibody analysis of keratin expression in epidermal diseases: a 48- and 56- kdalton keratin as molecular markers for hyperproliferative keratinocytes. *J Cell Biol* 1984; **98:** 1397–406.

8 Hunter I, Skerrow D. The effect of increased tissue turnover on the keratinization of human epidermis. *Biochim Biophys Acta* 1981; **674:** 155–9.

9 Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970; **277:** 680–5.

10 Skerrow D. The proteins of epidermis in relation to normal and abnormal keratinization. In: *The Ichthyoses* (Marks R, Dykes PJ, eds). Lancaster: MTP Press, 1978; 43–50.

11 Hunter I, Skerrow D. The proteins of living psoriatic epidermis. *Biochim Biophys Acta* 1981; **714:** 164–9.

12 Bowden PE, Cunliffe WJ. Modification of human prekeratin during epidermal differentiation. *Biochem J* 1981; **199:** 145–54.

13 Bowden PE, Quinlan RA, Breitkreutz D *et al.* Proteolytic modification of acidic and basic keratins during terminal differentiation of mouse and human epidermis. *Eur J Biochem* 1984; **142:** 29–36.

14 Mansbridge JN, Knapp AM. Changes in keratinocyte maturation during wound healing. *J Invest Dermatol* 1987; **89:** 253–63.

15 Löning T, Staquet M-J, Thivolet J, Seifert G. Keratin polypeptide distribution in normal and diseased human epidermis and oral mucosa. *Virchows Arch (A)* 1980; **388:** 273–88.

16 Bernard BA, Robinson SM, Vandaele S *et al.* Abnormal maturation pathway of keratinocytes in psoriatic skin. *Br J Dermatol* 1985; **112:** 647–53.

17 Bell CM, Skerrow CJ. Lectin binding to psoriatic epidermis. *B J Dermatol* 1985; **113:** 205–12.

18 Dale BA, Holbrook KA. Developmental expression of human epidermal keratins and filaggrin. In: *The Molecular and Developmental Biology of Keratins* (Sawyer RH, ed.) Current Topics in Developmental Biology, Vol. 22. Orlando: Academic Press, 1987; 127–51.

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Sex differences in susceptibility to development of contact hypersensitivity to dinitrochlorobenzene (DNCB)

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SUMMARY

We have investigated the differences between the sexes in the development of contact sensitivity induced by dinitrochlorobenzene (DNCB). Ten male and 12 female subjects were sensitized with DNCB (30 μg applied on a 1 cm patch test disc) and challenged 1 month later with doses of 8·8, 12·5, 17·7 and 25 μg. The responses were measured after 48 h as increase in skinfold thickness with Harpenden callipers. Females showed a larger response at all challenge doses studied, and the slope of the log-dose response curve was significantly steeper in females. We conclude that there are significant differences in delayed type hypersensitivity between males and females.

Despite increasing knowledge of the cellular immune mechanisms involved in delayed type hypersensitivity, the factors determining the induction and expression in man are poorly understood. For instance, it is not possible to predict why, given equal exposure to antigens, some people develop contact sensitivity and others do not. It is well known that more females than males present with problems relating to contact sensitivity. This is confirmed by our statistics: analysis of patch test results over a 5-year period (1981–1986) showed that the ratio of females to males with one or more positive patch test results was >3:1. The marked preponderance of females with many diseases thought to be related to immune dysfunction suggests that there may be significant differences in immune responses between males and females. That more females show positive patch test results cannot alone be taken to demonstrate differences in delayed type hypersensitivity between the sexes because there may be sex-related differences in exposure to antigen, the populations from which the positives are taken are not defined, and there may be bias in referral. In order to study this problem we examined the response of males and females to dinitrochlorobenzene (DNCB).

We have previously shown that sensitivity to DNCB, a known potent immunogen, follows predictable log-dose characteristics.[1] We, therefore, sensitized male and female subjects with a standard dose of DNCB and determined their response to challenge by measuring increase in skinfold thickness with Harpenden callipers as previously described.[1]

METHODS

Subjects

Ten male and 12 female patients newly referred to the skin department aged between 18 and 65 were asked to participate in the study. All gave fully informed consent and ethical committee approval was obtained. Patients with inflammatory dermatoses, or patients referred with possible contact sensitivity were excluded. No patients were included who were receiving any drugs or treatment such as topical steroids or ultraviolet radiation known to interfere with the development or expression of contact sensitivity.

Sensitization and challenge

Subjects were sensitized with 30 μg of DNCB (24 μl of 0·125% DNCB in acetone) on a 1 cm diameter patch test pad (Al-test) held in place on the medial side of the thigh for 48 h. One month later, sensitivity was determined by measurement of responses to a graded series of challenge doses applied simultaneously. Subjects were challenged with four doses of DNCB, increasing by a factor of $\sqrt{2}$ (8·8, 12·5, 17·7, 25 μg) placed on Al-test pads on the inner aspect of the upper arm. Skinfold thickness was measured at each site in duplicate immediately pre and 48 h post challenge. In order to assess the effect of leaving the challenge dose on for different periods of time in 14 subjects the challenge dose was left on 6 h on one arm and 12 h on the other. There was no difference in the results obtained and, therefore, the mean of these individual readings was used. In the remaining subjects the challenge was left on 6 hours. The responses to challenge were expressed as increase in skinfold thickness (final thickness minus initial thickness) against log-challenge dose. The results of challenge were plotted as log–dose–response curves which were compared by a split-unit analysis of variance.

RESULTS

Two male patients who failed to become sensitized were excluded from subsequent analysis. The results for males and females were plotted as change in skinfold thickness against log-challenge dose and are shown in Figure 1. As expected, in both sexes there was evidence of a linear relationship between response measured as change in skinfold thickness and the log-challenge dose. There was an obvious difference between the sexes with females showing a larger increase in skinfold thickness at all challenge doses. At the smallest challenge dose (8·8 μg) the mean response was 1·6 mm in females as compared with 0·6 mm for males and at the largest challenge dose (25 μg) the increase in skinfold thickness was 3·9 mm and 2·0 mm respectively, a mean difference of 1·9 mm. The curves were clearly not parallel which demonstrates that the magnitude of the difference between the sexes was not constant but increases with the challenge dose i.e. there is a strong dose by sex interaction ($P = 0{\cdot}008$).

DISCUSSION

We have confirmed that the response measured as change in skinfold thickness to challenge with DNCB follows predictable log-dose characteristics. In addition we observed a significant difference between male and female subjects, females showing a larger response at all challenge

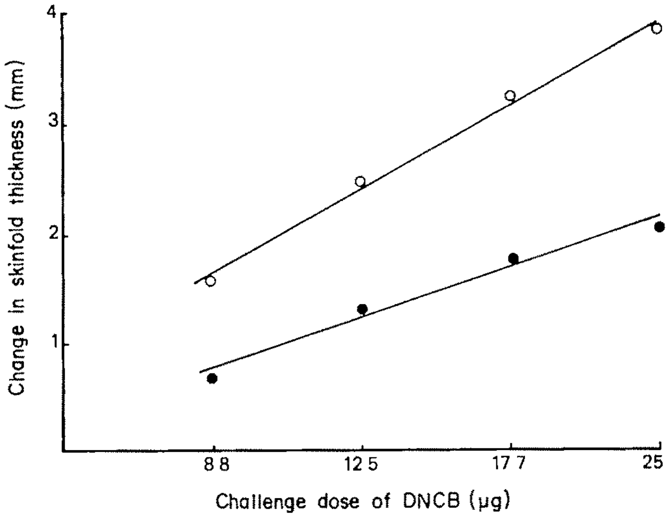

FIGURE 1. Responses to challenge with DNCB in male (●) and female (○) subjects sensitized with 30 µg of DNCB

doses. There was evidence of a strong sex by dose interaction with the dose-response curve being steeper in females than males. Although the patients studied were not chosen at random from the population, it is unlikely the group will be biased with respect to the development of contact hypersensitivity as patients referred with a history of contact allergy were excluded. Because subjects with one positive sensitivity have an increased susceptibility to the development of another[2] and as the prevalence of nickel sensitivity is greater in females it is possible, since these subjects were excluded from the study, that we have underestimated the magnitude of the differences in response to DNCB between the sexes.[2] Similarly, because the two male patients who apparently did not sensitize were excluded from the analysis, it is likely that the difference we report is an underestimate.

The results are in keeping with our clinical observation that females are more likely to develop contact sensitivity than males. The use of an antigen such as DNCB allows us to control exposure to an antigen that is not possible with results obtained from conventional patch test readings where the possibility of previous differences in environmental exposure between the sexes cannot be excluded. Our results indicate that even if exposure to environmental antigens is the same, females are more likely to develop contact sensitivity. Earlier studies have shown conflicting results with Leyden and Kligman[3] failing to detect any difference in sensitization between men and women whereas Jordan and King[4] suggested that females were more prone to develop sensitization. Unfortunately, in these studies the methods were only semi-quantitative, no statistical analysis was presented and previous exposure to antigen was not controlled for. Studies in mice have shown sex differences in cell mediated hypersensitivity. Female mice rejected skin allografts quicker than males[5] and gonadectomy could reduce the magnitude of the difference. In male mice the response to the sensitizer oxazolone was reduced following orchidectomy.[6] However, in the guinea-pig these differences in contact sensitivity were not found.[7]

Our studies provide no information as to whether it is the afferent or the efferent limb of the immune response that is different in males and females. Little is known concerning the pharmacokinetics of DNCB and differences in penetration to the immunocompetent cells that present the antigen could account for the observed differences in sensitization. Koyama *et al.*[8] have reported sex differences in Langerhans cell numbers in mice, with females having a higher density than males. These differences have not been reported in human skin[9] nor have we observed alteration in the number of Langerhans cells between the sexes (Rees and Friedmann, personal observation). Bjornberg[10] failed to detect any sex differences in the response of the skin to irritants suggesting that the observed difference in response to DNCB is not due to differences in non-specific cutaneous inflammation between the sexes.

Whatever the explanation for the difference between the sexes, our results provide convincing evidence that males and females exposed to the same initial dose of an antigen respond significantly differently on challenge and that this difference is reflected in the different prevalence of contact sensitivity to environmental allergens.

REFERENCES

1 Friedmann PS, Moss C, Shuster S, Simpson J. Quantitative relationships between sensitising dose of DNCB and reactivity in normal subjects. *Clin Exp Immunol* (1983); **53:** 709–15.

2 Moss C, Friedmann PS, Shuster S, Simpson J. Susceptibility and amplification of sensitivity in contact dermatitis. *Clin Exp Immunol* (1985); **61:** 232–41.

3 Leyden J, Kligman A. Allergic contact dermatitis: Sex differences. *Contact Dermatitis* (1977); **3:** 333–6.

4 Jordan W, King S. Delayed hypersensitivity in females. *Contact Dermatitis* (1977); **3:** 19–26.

5 Graff RJ, Lappe MA, Snell GD. The influence of the gonads and adrenal glands on the immune response to skin grafts. *Transplantation* (1969); **7:** 105–11.

6 Castro JE. Immunological effects of orchidectomy. *Br J Urol* (1975); **47:** 89–95.

7 Magnusson B and Kligman A. *Allergic Contact Dermatitis in the Guinea-Pig*. Springfield, Illinois: Thomas, cited in Leyden J and Kligman A. Allergic contact dermatitis: Sex differences. *Contact Dermatitis* (1977); **3:** 333–6.

8 Koyama Y, Nagio S, Ohashi K *et al.* Sex differences in the densities of epidermal langerhans cells of the mouse. *J Invest Dermatol* (1986); **88:** 541–4.

9 Berman B, Chen VL, France DS *et al.* Anatomical mapping of epidermal Langerhans cell densities in adults. *Br J Dermatol* (1985); **113:** 553–8.

10 Bjornberg A. Skin reactions to primary irritants in men and women. *Acta Derm Venereol (Stockh)* 1975; **55:** 191–4.

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Warts in fish handlers

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SUMMARY

Fish handlers frequently suffer from hand warts. The clinical form and HPV type in these lesions were studied. Eleven individuals (10 fishmongers and one fisherman) with multiple hand warts were examined clinically and samples from their warts examined by Southern blot and reverse blot analysis. Clinically, with one exception, the warts were of the common type. HPV DNA was detected in all but one individual. HPV4 was found in one sample, HPV1 related virus in three, a virus hybridizing with both HPV27 and HPV2 in five (four individuals) and HPV7 in seven (six individuals). More than one type was detected in four individuals. HPV7 infection was related to the greater length of time spent in handling fish. These findings indicate that HPV7 is not, as was previously thought, found exclusively in those handling butcher meat and suggest that environmental conditions may be a factor in the clinical manifestation of HPV7 infection. The exact nature of a virus designated HPV2/27 and the significance of its presence in these fish handlers remains uncertain.

It has been known for more than 20 years that people working with fresh or frozen meat, poultry or fish, suffer from warts more frequently than do controls.[1,2] In one study of 430 meat cutters, 44% had warts.[3] Later studies confirmed this high prevalence of hand warts.[4–9] The question was raised in 1969 as to whether meat, poultry or fish (or all of these) might be the source of the virus responsible for warts in these individuals. Since that time it has become possible to identify many different human papillomaviruses (HPV) occurring in warts. Whereas common warts are usually associated with HPV2, 4 or 28,[10] a number of warts on butcher's hands were found to contain an HPV type not previously detected.[6] This HPV type occurred in 38 of 104 butchers (36·5%) and was characterized as HPV7.[11] The failure of HPV7 to cross hybridize with bovine papillomaviruses (BPV 1–4) excludes the possibility that it is related to BPV.[12,13] Studies with other animal papillomaviruses have not been reported.

Another possible explanation for the high prevalence of warts in meat handlers is that the skin of their hands is chronically macerated and traumatized in the course of their work, facilitating infection with particular wart viruses, in this case HPV7. An additional method of eliminating butcher meat as a possible source of HPV7 infection is to study a group of people who have

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similar working conditions but handle fish only. Litt[3] and Marshall[4] found that people handling fish often suffered from extensive warts. We had previously observed a clinical similarity between hand warts of butchers and fishmongers. We investigated a group of fish handlers to determine the types of warts present and to look for the presence of HPV7, as this did not appear to have been done previously.

METHODS

Patients

Questionnaires were sent to 93 wholesale and retail fish merchants with entries in the Edinburgh commercial telephone directory in order to find fish handlers with hand warts. We also included any fish handlers already attending the wart clinic at the Edinburgh Royal Infirmary.

Virological methods

Samples from hand warts were obtained either by paring or curettage. Parings from several warts in the same patient were pooled. DNA was extracted and digested by restriction endonucleases *Hind* II/III, *Pst* I, *Ava* II and *Hpa* I, Southern blotted and hybridized sequentially with clones of HPV1-4, 7, 10 and 27,[13-15] washed at $T_m - 35°C$ and then at $T_m - 15°C$. Details of the method have previously been described.[16] In addition, four samples were tested by a reverse Southern blot technique as described by de Villers *et al.*[17] in which the DNA extracted from the clinical specimen is radioactively labelled and hybridized to a filter on which a panel of HPV clones, released from their vectors, has been blotted. In this case, clones of HPVl-27, 30, 31 and 33-38 were used.

RESULTS

Forty-one firms replied to the questionnaire. Thirteen (38%) reported having employees with hand warts. We were able to examine and take samples from 10, each from a different firm, and from one commercial fisherman attending the wart clinic. The clinical details of these patients are shown in Table 1. There were six males and five females who all worked in water and were involved in catching, gutting, filleting or curing of fish, seven for more than 5 years. They all had multiple warts which were, with one exception, of the common type (Fig. 1). The back of the fingers was the most commonly affected site. The duration of the warts varied from 3 months to 24 years (mean 2 years). Eight had contact with others with warts at work, four had contact with warts at home but only one of these did not have contacts at work also. Only four had a past history of warts.

Virological results

The results of the Southern blot analysis of 12 samples from the 11 patients are shown in Figure 2, and listed in Table 2. No DNA was detected in the sample from one patient, a single type of HPV DNA was found in six, and two types in four patients. Amongst the samples, HPV7 was detected in 7, an HPV hybridizing with HPV2/27 but with unusual bands in five, an HPV hybridizing to HPV1 at low stringency only in three and HPV4 in one.

One HPV7 positive sample was analysed by 'reverse Southern blotting' and hybridized only with cloned HPV7 under conditions of high stringency, thus confirming HPV7 positivity (Fig. 3). The restriction enzyme pattern also corresponded with those published for HPV7 with *Pst* I, *Ava* II and *Hpa* I,[18] but not those for *Hind* II/III.[6] Three samples which had shown an unusual

TABLE 1. Clinical details of fish handlers with hand warts

	Patient no.										
	1	2	3	4	5	6	7	8	9	10	11
Sex	F	F	M	F	F	M	M	M	M	F	M
Length of time at work (years)	3	2	20	25	2	31	18	1·5	8·5	8	6
Wart contacts at work	+	+	+	–	+	+	–	–	+	+	+
Wart contacts at home	–	+	–	+	+	+	–	–	–	–	–
Past history of warts	+	+	+	–	–	–	?	+	–	–	–
Duration of warts (years)	2	?	3	2	1·5	3	20	0·3	7	0·5	2·4
Type of warts	c	c	p	c	c	c	c	c	c	c	c
Distribution:											
Fingers—											
Dorsal surface	—	m	m	m	m	m	m	m	m	—	m
Palmar surface	m	–	–	–	–	m	–	m	m	s	–
Hands—											
Palms	–	–	–	m	–	–	–	m	–	s	–
Dorsal surface	–	–	m	–	–	–	–	–	–	–	–

c = common wart, p = plane wart, s = single wart, m = multiple warts

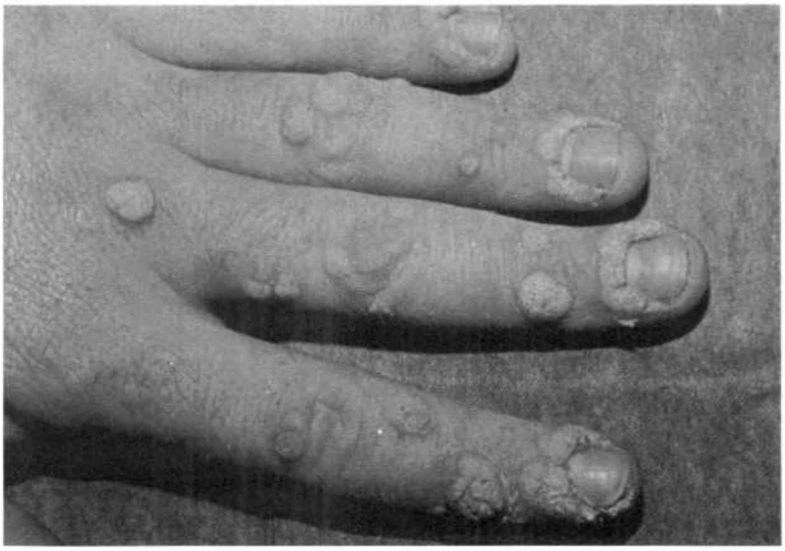

FIGURE 1. Common warts on the hands of a fishmonger.

restriction enzyme pattern when hybridized with HPV2 were also tested by reverse Southern blotting under stringent conditions. They showed hybridization with clones of both HPV2 and 27 (Fig. 4). Subsequently hybridization with HPV27 by conventional Southern blotting gave strong positive signals. However, as the restriction enzyme pattern for HPV27 is not yet published, we have designated these isolates HPV2/27.

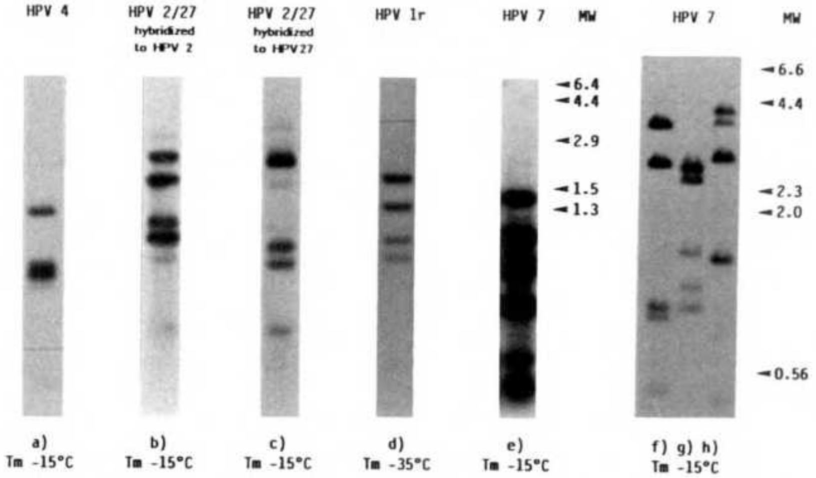

FIGURE 2. Southern blot analysis of warts from fish handlers: Total cellular DNA extracted from wart samples, digested with different restriction endonucleases (lane a–e, *Hind* II/III, lane f, *Pst* I, lane g, *Ava* II and lane h, *Hpa* I), and hybridized to different HPV clones as indicated at the top of each lane. Molecular weights are indicated in kilo basepairs (kbp).

TABLE 2. Virological results

		HPV DNA Detected			
Patient No.	Sampling* method	HPV7	HPV4	HPV1 related;†	HPV2/27‡
1	p	–	–	–	–
2	p	–	+	–	–
3	p	–	–	–	+
4	p	+	–	–	
5	p	–	–	–	+
6	c	+	–	–	–
7	p	+	–	+	–
8	p		–	+	+
9a	c	+	–	–	+
b	c	+	–	–	+
10	p	+	–	–	–
11	c	+	–	+	–
Total		7	1	3	5

*p = pooled parings from more than one wart.

c = curette specimen.

† = HPV1 related virus which hybridized with HPV1 clone under conditions of low stringency only

‡ = HPV2/27—an HPV type that cross hybridized with both HPV2 and HPV27 under conditions of high stringency.

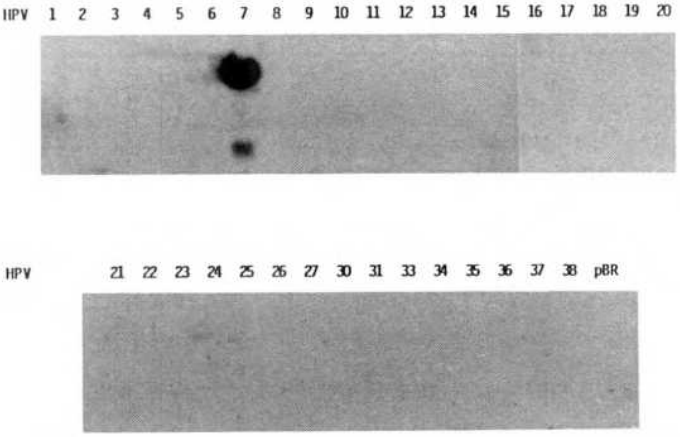
HPV 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
HPV 21 22 23 24 25 26 27 30 31 33 34 35 36 37 38 pBR

FIGURE 3. Reverse Southern blot of an HPV7 induced wart from a fishmonger: Total cellular DNA extracted from a fishmonger's wart was radiolabelled and hybridized to a panel of HPV clones (HPV1–27, 30, 31, 33–38). Under conditions of high stringency (Tm − 15°C) only cross hybridization with the HPV7 clone was detected.

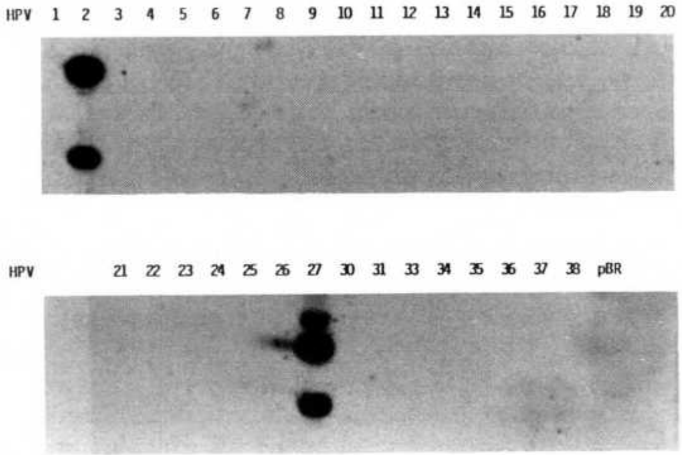
HPV 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
HPV 21 22 23 24 25 26 27 30 31 33 34 35 36 37 38 pBR

FIGURE 4. Reverse Southern blot of HPV2/27 induced wart from a fishmonger: Total cellular DNA from three fishmongers' warts (patient numbers 3, 5 and 8) and extracted and analysed by reverse Southern blot. Under conditions of high stringency (Tm − 15°C) hybridization was detected to HPV2 and 27 only.

DISCUSSION

No conclusion as to the prevalence of warts in fish handlers nor how this compares with that amongst meat handlers can be drawn from this study because of the small numbers involved.

The clinical impression of the similarity of the warts in fish and meat handlers was upheld in that common warts predominated. Plane warts and deep palmar warts (HPV1 type) have also been reported in butchers.[6] One fish handler had plane warts and an HPV1 related virus was found in the warts of three, although these did not have the classical appearance . There can be no doubt that our HPV7 isolates, which were all of the same restriction enzyme type, were true

HPV7, as under conditions of high stringency, whilst hybridizing strongly with the HPV7 clone, they did not hybridize with the other HPV types tested (HPV1–6, 8–27, 30, 31, 33–38). Whilst differing slightly from those reported by Jablonska *et al.* for *Hind* II/III, the restriction enzyme pattern of these isolates agreed with those of de Villers *et al.*[18] for *Pst* I, *Ava* II and *Hpa* I. Jablonska has pointed out that some heterogenicity exists amongst different HPV7 isolates.[6]

It could be argued that the HPV7 in these fish handlers originated in the fish. Fish occasionally develop papillomata[19] and there is some evidence that these may be virally induced.[20,21] Papilloma viruses are strictly species specific so it is unlikely that both fish and land animals would be infected with the same type, or indeed that HPV7 should be anything other than a true papilloma virus. Other indications that this is so were presented by Oltersdorf *et al.*, who obtained some cross hybridization with the 'mucosal' HPV types (HPV6, 11, 16, 18 and 31)[13] and recently by de Villers *et al.* who reported the detection of HPV7 in the filiform warts of two non-butchers.[18] We have also detected HPV7 in a filiform wart in a non-meat non-fish handler (Rüdlinger *et al.*, unpublished).

The role played by chronic skin maceration, present to a marked degree in those who handle fish or meat, in promoting infectivity with HPV7 can only be conjectural. However, it may be of significance that in these fish handlers there was a relationship between the length of time in the occupation and infection with HPV7.

It is of interest that two other unusual HPVs were detected in our fish handlers, HPV1 (related) and HPV2/27. It is surprising that no typical HPV2 was found as this is the type most usually associated with common warts.[10] However, the isolate we designated HPV2/27 whilst hybridizing with HPV2 showed an unusual restriction pattern and subsequently hybridized to HPV27 in both cases under conditions of high stringency. It is known that HPV27 will cross-hybridize with HPV2.[22] The restriction enzyme pattern of HPV27 has not yet been published so it is not possible to identify the isolate further at present. The significance of its presence remains to be investigated.

This study has demonstrated that HPV7 can occur in those who handle fish as well as in those who handle butcher meat. It suggests that the virus is unlikely to originate from animal sources and that environmental factors may be a necessary precondition for clinical HPV7 infection.

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REFERENCES

1 Bosse K, Christophers E. Beitrag zur Epidemiologie der Warzen. *Hautarzt* 1964; **15**: 80–6.

2 Mergler D, Vezina N, Beauvais A. Warts among workers in poultry slaughterhouses. *Scand J Work Environ Health* 1982, **8**: 180–184.

3 Litt J. Warts in meat-cutters. *Arch Dermatol* 1969; **100**: 773.

4 Marshall J. *Skin Diseases in Africa: An Essay in Epidemiology*. Cape Town: Maskew Miller, 1964, **14**: 44

5 DePeuter M, DeClercq B, Minnette A. An epidemiological survey of virus warts of the hands among butchers. *Br J Dermatol* 1977; **96**: 427–31.

6 Jablonska S, Obalek S, Favre M *et al.* The morphology of butchers' warts as related to papillomavirus types. *Arch Dermatol Res* 1987; **279:** 66–72.

7 Taylor SW. A prevalence study of virus warts on the hands in a poultry processing and packing station. *J Soc Occup Med* 1980; **30:** 20–23.

8 Wall LM, Oakes D, Rycroft RJ. Virus warts in meat handlers. *Contact Dermatitis* 1981; **7:** 259–67.

9 Finkel ML, Finkel DJ. Warts among meat handlers. *Arch Dermatol* 1984; **120:** 1314–7.

10 Pfister H. *Current papillomavirus classification scheme.* VI International Papillomavirus Workshop, Washington DC 1987; 1–10.

11 Orth G, Jablonska S, Favre M *et al.* Identification of papilloma viruses in butchers' warts. *J Invest Dermatol* 1981; **76:** 97–102.

12 Ostrow RS, Krzyzek R, Pass F, Faras AJ. Identification of a novel human papilloma virus in cutaneous warts of meat handlers. *Virology* 1981; **108:** 21–7.

13 Oltersdorf T, Campo MS, Favre M *et al.* Molecular cloning and characterization of human papillomavirus type 7 DNA. *Virology* 1986; **149:** 247–50.

14 Ostrow RS, Zachow K, Watts S *et al.* Characterization of two HPV-3 related papillomaviruses from common warts which are distinct clinically from flat warts or epidermodysplasia verruciformos. *J Invest Dermatol* 1983; **80:** 436–40.

15 Ostrow RS, Zachow K, Weber D *et al.* Presence and possible involvement of HPV DNA in premalignant and malignant tumors. UCLA Symposia on Molecular and Cellular Biology, New Series. *Papillomaviruses: Molecular and Clinical Aspects.* (Howley PM, Broker TR eds), New York: ARLiss 1985; **32:** 101–22.

16 Rudlinger R, Smith IW, Bunney MH, Hunter JAA. Human papillomavirus infections in a group of renal transplant recipients. *Br J Dermatol* 1986; **115:** 681–92.

17 deVilliers E-M, Schneider A, Gross G, zur Hausen H. Analysis of benign and malignant urogenital tumors for human papillomavirus infection by labelling cellular DNA. *Med Microbiol Immunol* 1986; **174:** 281–6.

18 deVilliers E-M, Neumann C, Oltersdorf T *et al.* Butcher's wart virus (HPV7) infections in non-butchers. *J Invest Dermatol* 1987, **87:** 236–8.

19 Sundberg JP. Papillomavirus infections in animals. In: *Papillomaviruses and Human Disease.* (Syrjaenen K, Gissman L, Koss LG eds), Springer-Verlag, Berlin, Heidelberg, New York, 1987; 40–103.

20 Bylund G, Valtonen ET, Niemela E Observations on epidermal papillomata in wild and cultured Atlantic salmon *Salmo salar L.* in Finland. *J Fish Dis* 1980; **3:** 525–8.

21 Wellings, SR, Chuinard RG. Epidermal papillomas with virus-like particles in flathead sole *Hippoglossoides elassodon. Science* 1964; **146:** 932–4.

22 Pfister H, Krubke J, Dietrich W *et al.* Classification of papillomaviruses—mapping the genome. In: *Papillomaviruses.* Ciba Foundation Symposium 120. Chichester: Wiley & Sons, 1986; 5.

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The significance of the Darier-like solar keratosis and acantholytic change in preneoplastic lesions of the epidermis

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SUMMARY

Fifty-two lesions of solar keratosis from 40 patients were studied prospectively for the presence of Darier-like changes and acantholysis. Twenty-seven per cent of specimens showed some degree of these changes. The affected lesions tended to be larger and were less common on the hands, but were otherwise clinically indistinguishable from other solar keratoses. Histologically, the specimens with Darier-like changes were significantly thicker and more dysplastic. The autoradiographic labelling index was not different for the lesions with these changes indicating similar cell kinetic characteristics.

It is suggested that acantholytic separation of epidermal cells within a solar keratosis may signify that the lesion has a greater invasive potential than a solar keratosis not showing this change.

The occurrence of cleft formation, or acantholysis, within the lower part of the dysplastic epidermis of a solar keratosis (SK) may be an unobtrusive isolated feature or may be accompanied by the extensive separation of a sheet of dysplastic cells that appear to have migrated around adjacent adnexal structures and uninvolved interfollicular epidermis. When these changes occur the terms Darier-like solar keratosis (DLSK) or acantholytic solar keratosis are sometimes applied. This apparent increased migratory potential of dysplastic tissue may be thought to indicate an increased invasive potential compared to the usual SK. However, there is no data on this issue. In order to characterize the significance of this Darier-like change (DLC), we have performed a prospective study in which excision and biopsy specimens of SKs have been studied to determine the incidence of these changes and to learn whether the phenomenon has any clinical significance or biological importance.

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METHODS

Patients

The subjects included in the study were patients attending the Dermatology Department for treatment of warty lesions that were diagnosed clinically as SKs. For the purpose of this study, SK was defined as a hyperkeratotic plaque or nodule in a light-exposed area with the histological features of epidermal dysplasia and dyskeratosis without frank infiltration of the underlying dermis. Forty patients were studied who had a total of 52 lesions removed that were histologically confirmed as SK. A detailed clinical history was taken from each patient, including details of the duration of the lesion(s), previous treatments, any previous history of skin disorder as well as details of any relevant medical history. The clinical examination included a record of the clinical appearance of the lesion, the diameter of the lesion and the presence or absence of other solar-induced changes in the skin. The lesion was then biopsied or excised after infiltration around the lesion with 1% lignocaine as a local anaesthetic.

Histology

The specimen was divided in half and in all cases one half was immediately fixed in 10% buffered formol saline solution and subsequently processed for routine light microscopy by staining 5 μm paraffin wax-embedded sections with haematoxylin and eosin. At least four sections of each specimen were examined for evidence of acantholysis, suprabasal cleft formation or signs of separation of cells from the main body of the epidermis. The degree of DLC was assessed and recorded on a visual analogue scale (VAS). In this technique a point is marked on a 10-cm line to indicate the intensity of the parameter under assessment, one end of the line being marked '0' and indicating the absence of the characteristic and the other end at 10 cm indicating the maximum degree that could be expected. Each specimen was also assessed for the degree of epidermal dysplasia, the degree of inflammation, the degree of epidermal

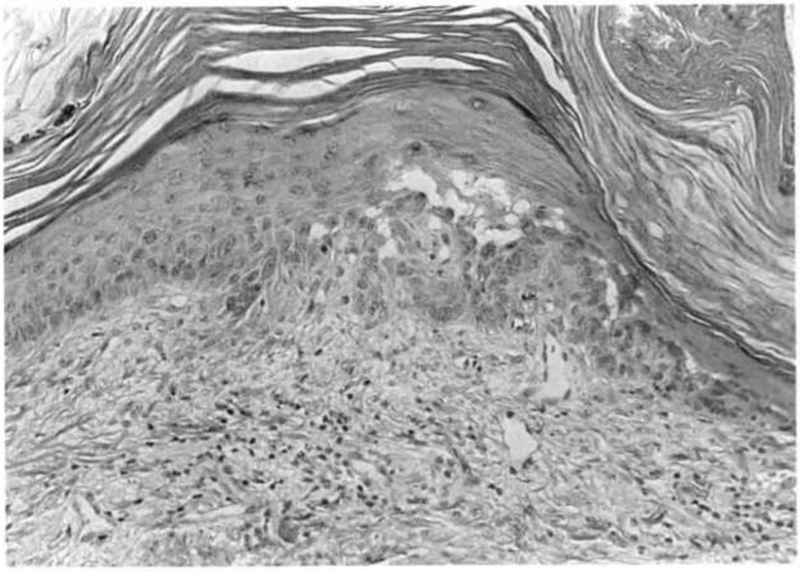

FIGURE 1. Darier-like change in a solar keratosis. A cleft has appeared above some proliferated abnormal basal epithelium (H&E; original × 120).

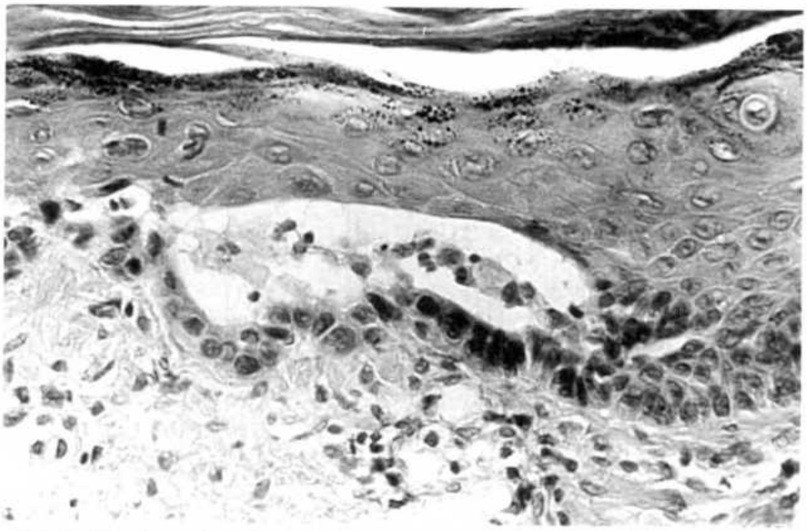

FIGURE 2. Darier-like change in a solar keratosis. Note the marked cleft formation. (H&E; original × 250).

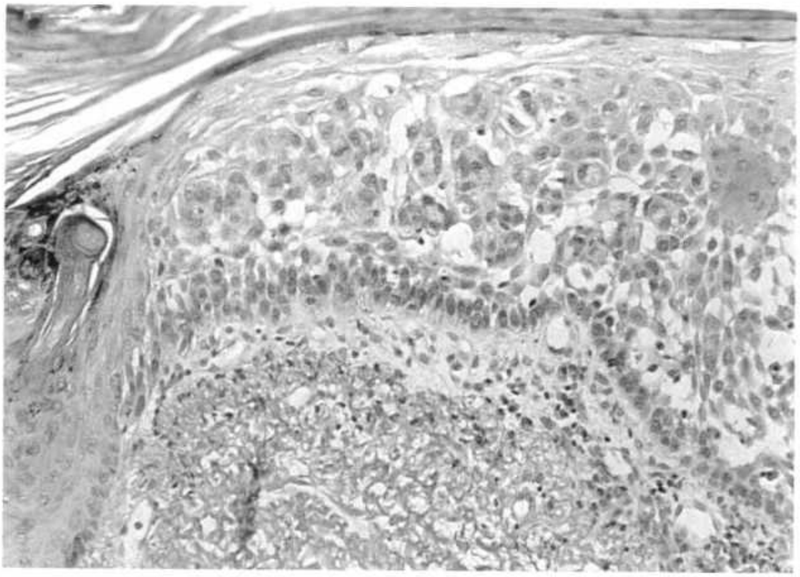

FIGURE 3. Marked epidermal vacuolation in a solar keratosis. (H&E; original × 120)

thickening and the degree of solar elastotic change seen in the underlying dermis on separate VAS rating scales. Two sets of observations by each observer were made on every specimen to test the validity of this method of assessment and there was good correlation between each pair of results (using Spearman's rank correlation coefficient $P < 0.05$). Visual analogue scale assessments have been used previously by our group to quantify the degrees of dysplasia and other features of solar keratoses.[1] They were found to be reproducible and correlated to objective measures of dysplasia.[2]

TABLE 1. Main clinical features of solar keratoses

	Solar keratosis with Darier-like change	Solar keratosis without Darier-like change	Statistical significance of differences
n	14	38	
Mean age (range) (years)	71·7 (54–88)	70·7 (37–86)	NS*
Sex ratio	5M·9F	19M:19F	NS**
Mean diameter (range) (mm)	6·5 (2–20)	4·6 (1–20)	$P < 0{\cdot}05$*
Site	Number (%)	Number (%)	
Scalp	0 (0)	3 (7·9)	NS**
Ear	1 (7·1)	3 (7·9)	NS**
Face	12 (85·7)	20 (52·6)	$P < 0{\cdot}05$**
Hand	1 (7·1)	10 (26·3)	$P < 0{\cdot}05$**
Leg	0 (0)	2 (5·3)	NS**

* Mann-Whitney *U*-test.
** χ^2-test.

TABLE 2. Visual analogue scale scores (cm) and labelling indices

	Solar keratosis with Darier-like change		Solar keratosis without Darier-like change		Statistical significance of difference
VAS scores for:	Median	Range	Median	Range	
Dysplasia	6·0	1·8–9·5	2·8	0·5–9·3	$P < 0{\cdot}05$*
Inflammation	3·7	0·4–9·1	2·0	0·2–8·1	NS*
Thickening	5·1	0·9–9·1	3·2	0·2–9·7	$P < 0{\cdot}05$*
Elastosis	6·4	1·8–8·2	4·5	1·0–8·7	NS*
Labelling index (%)	9·5 (mean)	9·1–11·3	11·3 (mean)	4·5–20·8	NS

*Mann-Whitney *U*-test.

Twenty-three specimens were investigated to determine whether acantholytic dysplastic epidermal tissue had particular cell kinetic characteristics. Fragments of the lesions were placed in Eagle's minimum essential medium and incubated for 4 h in 95% O_2 and 5% CO_2 in a shaking incubator at 37°C in the presence of tritiated thymidine (18 Ci/mmol) at a concentration of 2·5 μCi/ml. Autoradiographs were prepared using the standard dipping film technique and epidermal labelling indices determined by expressing the number of basal and suprabasal epidermal cells autoradiographically labelled with more than six grains over each nucleus as a function of the total number of basal cells.

RESULTS

Histological examination of the 52 specimens studied showed some degree of DLC in 14 specimens (27%) (Figs 1 and 2). Observation of the lesions showing extensive acantholytic

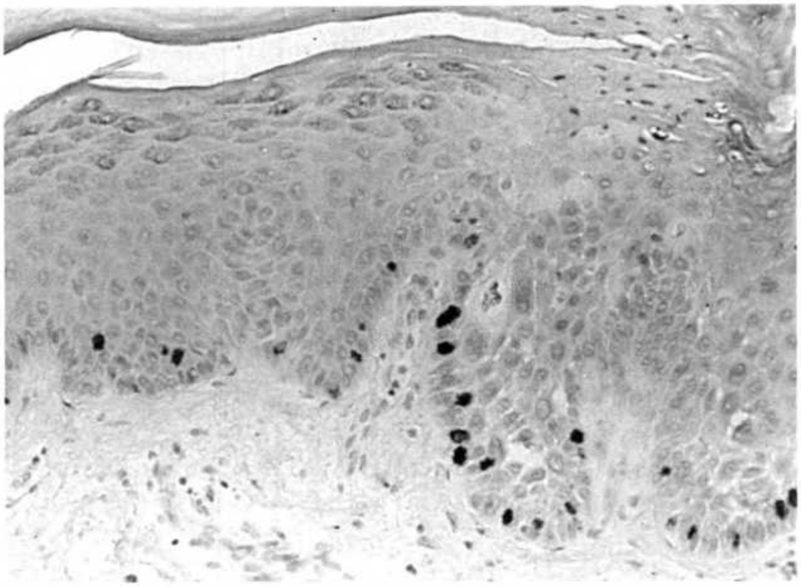

FIGURE 4. Autoradiograph after incubation with tritiated thymidine showing labelled cells in the basal layers of a solar keratosis. (H&E; original × 120).

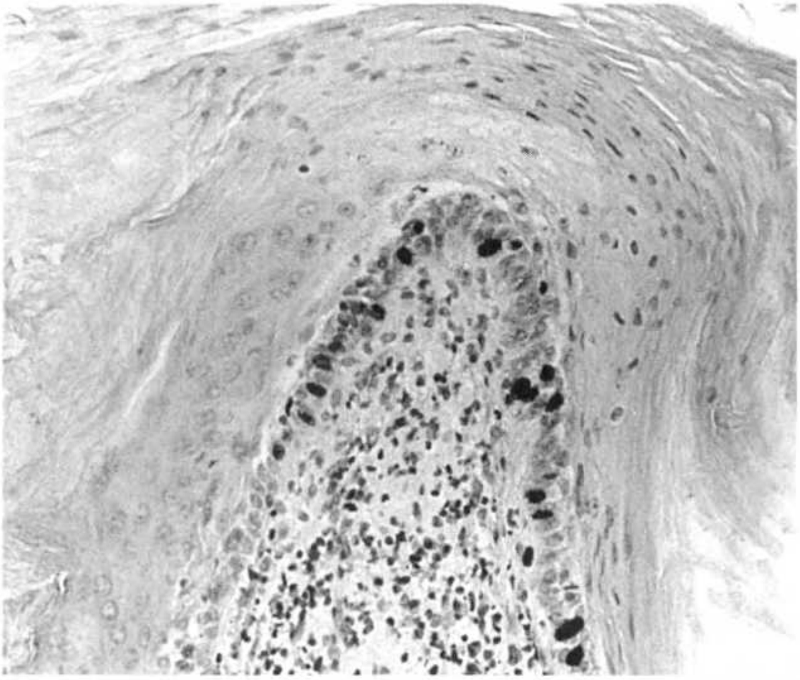

FIGURE 5. Autoradiograph after incubation of a solar keratosis in the presence of tritiated thymidine. Note the presence of autoradiographically labelled cells in the Darier-like portion. The degree of labelling is similar to that in the solar keratosis seen in Fig. 4. (H&E; original × 120).

changes often revealed an unusual morphological feature in which the abnormal basal dysplastic sheet of cells appeared separated from the epidermal cells above by both vacuolated epidermal cells and oedematous spaces between cells (Fig. 3). This oedematous or spongiotic appearance occurred in 11 of our 14 specimens showing DLC and not at all in SKs not showing the DLC. In four of the 11 showing this alteration, the change was marked with several areas of the abnormal epidermis being involved. The degree of spongiosis and epidermolytic changes appeared to be much the same as the amount of DLC that was present.

There was no difference in the clinical appearance of the lesions which could be categorized as DLSK and those keratoses that could not. The lesions in both categories were variously described as scaly, flat, crusted or warty. There were no differences in the ages or ratio of male to female amongst the patients that had lesions with DLC and those that did not. When the sites of the lesions were compared it was found that lesions with DLC occurred less frequently on the dorsum of the hands than would have been expected. The DLSK lesions were significantly larger than non-DLSK lesions (Table 1).

When the histological appearances of the lesions with and without evidence of acantholysis were compared, there was no statistically significant difference in the degree of inflammation or solar elastosis although the lesions with DLC were significantly thicker and more dysplastic. The autoradiographic labelling indices were not significantly different for lesions with and without DLC (Table 2). The labelled cells did not appear to be localized preferentially in the acantholytic portions of the lesion (Figs 4 and 5).

DISCUSSION

The occurrence of isolated tumours resembling lesions of Darier's disease was previously described as 'warty dyskeratoma' but Jablonska and Chorzelski[3] recognized that at least some of these lesions represented a variant of solar keratosis in which clefts occurred between epidermal cells. This variety of solar keratosis is mentioned in some textbooks of dermatopathology as 'carcinoma segregans',[4] but little has been published concerning the frequency of this change or its clinical or biological significance. Migration of sheets of neoplastic cells within the epidermis, formation of clefts between different populations of cells, and acantholysis represent loss of normal mechanisms of intercellular adhesion from malignant or premalignant keratinocytes. Our finding of DLC in 27% of cases studied prospectively contrasts with the only other published study[5] in which a retrospective survey found acantholysis alone in 4·4% of biopsy specimens reviewed, but no mention was made of any other of the features of DLC which might have been present. We believe that the reason for the larger proportion of SKs that showed the change in our study is that we were strict in only accepting lesions into the series that were unequivocal SKs, and that we accepted lesions as having DLC that showed minor degrees of acantholysis as in Figure 2. There was no statistically significant difference in the age or sex ratio between patients with SKs showing DLC and those without. There appeared to be no difference between the two groups of patients when compared for duration of lesions or the degree of solar damage, although these parameters are difficult to quantify. However, we agree with the earlier observation[5] that DLC seems to be less common in SKs occurring on the dorsum of the hands. The finding that the DLC is associated with histologically thicker and more dysplastic lesions does suggest that these lesions may have more potential for invasive behaviour. This possibility is difficult to validate experimentally. The autoradiographic labelling index results revealed no difference between lesions with DLC and those without this change. We can only presume that the proliferative potential of the two types of lesion is similar.

In a recent study,[6] it was suggested that many SKs may resolve spontaneously. In the context of patient management, it is important to be able to predict whether some precancerous lesions are more likely to progress than others. Also, it has also been suggested[7] that acantholytic squamous cell carcinoma derived from solar keratoses are more likely to metastasize. The vacuolated and spongiotic appearance of the epidermis reported here seems likely to be due to the same or similar processes as that causing DLC, as the two processes often seemed to occur together. Ackerman and Reed[8] described an epidermolytic variant of SK in two patients and suggested that it was 'an analogue' of the acantholytic change. The photomicrographs published in their paper appear to be similar, but more dramatic examples of the vacuolated and spongiotic changes were represented in our slides.

The biological significance of the separation of epidermal cells in the lesions of SK, one from the other, is unclear. It may signify loss of junctional apparatus or specific attachment domains at the cell surface. It is not known how this relates to the development of progression from premalignant lesions of the epidermis, to frank malignancy with local invasion or metastasis. Further studies are intended.

REFERENCES

1 Tan CY, Marks R. Lichenoid solar keratoses — prevalence and immunological findings. *J Invest Dermatol* 1982; **79:** 365–7.

2 Barton S, Pearse AD, Marks R. Epidermal dysplasia and solar elastosis and their interrelationships. *G It Chir Dermatol Oncol* 1987; **2:** 435.

3 Jablońska S, Chorzelski T. Dyskeratoma and epithelioma (carcinoma) dyskeratoticum segregans. *Dermatologica* 1961; **123:** 24–37.

4 Mehregan AH. *Pinkus' Guide to Dermatohistopathology*, 4th edn., Norwalk: Appleton-Century-Crofts, U.S.A., 1986.

5 Carapeto FJ, Garcia-Perez A. Acantholytic keratosis. *Dermatologica* 1974; **148:** 233–9.

6 Marks R, Foley P, Goodman G *et al* Spontaneous remission of solar keratoses: the case for conservative management. *Br J Dermatol* 1986; **115:** 649–55.

7 Uchiyama N, Shindo Y. A case of acantholytic squamous cell carcinoma derived from the acantholytic type of solar keratosis. *J Dermatol (Tokyo)* 1986; **13:** 377–80.

8 Ackerman AB, Reed RJ. Epidermolytic variant of solar keratosis. *Arch Dermatol* 1973; **107:** 104–6.

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The usefulness of immunofluorescent tests in pemphigus patients in clinical remission

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SUMMARY

Direct and indirect immunofluorescent studies (DIF, IIF) were performed on 24 pemphigus vulgaris patients who were in a state of clinical remission. The tests were repeated after an interval of 6 months. All the patients were on maintenance therapy with oral prednisone. The DIF in eight patients showed negative results among whom seven remained negative. Six patients out of 24 showed weakly positive fluorescence and ten patients showed strong positive fluorescence. The IIF was negative in 17 patients and positive in seven patients who also showed positive DIF. During a follow-up period of 20 months, one of eight patients with negative DIF relapsed compared with two of six patients with weak positive DIF and five of 10 patients with strong DIF. Five patients with strong DIF for IgG also had C_3, of whom three relapsed, compared with five of 19 patients who were negative for C_3. Four of seven patients with positive IIF relapsed compared with four of 17 with negative IIF. It is suggested that repeated DIF tests in pemphigus patients, who are in clinical remission, may serve as an indicator for the immunological activity and be of help in the management of these cases.

Pemphigus vulgaris is regarded as an autoimmune disease in which autoantibodies are demonstrable in the epidermis by the direct immunofluorescent test (DIF) and in the serum by the indirect immunofluorescent test (IIF)[1]. The DIF is a very reliable diagnostic test for pemphigus vulgaris and can remain positive for many years after the disease has regressed.[2] The titre of antibodies in the sera of patients is in many instances proportional to the severity of the disease.[3] However, there are too many exceptions for the titre to be regarded as a reliable index for the severity of the disease,[4] and it cannot be used as a guide for therapeutic judgement.[5] The purpose of this study was to follow up pemphigus patients who were in clinical remission and to investigate the fate of both epidermal and circulating auto-antibodies by means of direct and indirect immunofluorescence techniques. We have tried to find out to what extent pemphigus

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antibodies correlate with the clinical remission and if epidermal IgG and C_3 deposits may serve not only as an indicator for immunological activity of the disease, but also for therapeutic considerations.

METHODS

Patients

Twenty-four patients were included in the study, thirteen males (age range 59–78 years) and eleven females (age range 32–87 years). In all the patients the diagnosis of pemphigus vulgaris (PV) was confirmed by histological and immunofluorescent criteria. Five patients out of 24 suffered from pemphigus vulgaris affecting the mucosae of the mouth. The duration of the disease was 2 to 11 years (average 6·1 years). At the beginning of the study all the patients were in a state of clinical remission of 6 months' to 4 years' duration. All of them were receiving maintenance therapy with oral prednisone, 5–20 mg daily or on alternate days.

All the patients were followed-up regularly every 2 months for 20 months after the starting point of the study. DIF and IIF tests were performed twice in each patient with an interval of 6 months between the first and second tests. In patients with repeated negative IF tests administration of prednisone was discontinued.

Immunofluorescent studies

DIF staining. Four millimetre punch biopsies were taken from the skin of upper back and frozen immediately. Cryostat sections were tested with fluorescein-conjugated antisera specific for human IgG and C_3 (Tago, Burlingame, CA, U.S.A.). The findings were graded according to the intensity of fluorescence: (−): negative staining, (+): weakly positive staining and (+ +): positive staining.

IIF test. All serum samples were examined using cryostat sections of normal human skin. Serum was initially diluted 1 to 10 in phosphate-buffered saline and further serial dilutions were made on positive sera. The sections were incubated with the patient's serum and then examined for the presence of IgG using the same fluorescein-labelled conjugates as in the DIF method.

RESULTS

The results of the DIF and IIF tests are detailed in Table 1. The initial immunofluorescence findings at the time of diagnosis are also given for purpose in comparison. All the patients remained in follow-up for 14 months after the repeated IF tests.

Eight patients out of 24 showed negative DIF. Seven out of the eight remained negative after 6 months and one became weakly positive. Seven patients remained in complete remission by the end of the study without any treatment. In one patient recurrence of lesions was observed after 1 year, following withdrawal of treatment.

Six patients showed weakly positive DIF. After 6 months four remained weakly positive, one became negative and one positive. At the end of the study four patients were still in complete remission. Two patients relapsed clinically after 6 and 8 months after the repeated IF tests.

Ten patients showed positive DIF. In five patients C_3 deposits were also detected. At the second time of testing, eight patients out of the 10 remained positive and two showed weakly positive DIF. Five patients developed recurrence of lesions and in the second test three of them

TABLE 1. Results of immunofluorescent studies

	Initial findings			IF findings during remission					
	DIF		IIF	DIF		IIF	DIF		IIF
	At diagnosis			I			II		
Patient no.	IgG	C_3	Titre	IgG	C_3	Titre	IgG	C_3	Titre
*1	+ +	+	1:40	+	–	—	+	–	—
2	+ +	–	—	+	–	—	+	–	—
3	+ +	–	1:20	–	–	—	–	–	—
*4	+ +	+ +	1:320	+ +	–	1:10	+	–	1:20
*5	+ +	+	—	+ +	+ +	1:20	+ +	+ +	1:40
6	+ +	+ +	—	–	–	—	–	–	—
7	+ +	+	—	+ +	+	1:40	+ +	–	1:20
*8	+ +	+ +	nd	+	–	—	+	–	—
9	+ +	+ +	nd	+ +	–	—	+	–	—
*10	+ +	–	—	+ +	–	1:10	+ +	–	—
11	+ +	+ +	1:80	–	–	—	–	–	—
12	+ +	+	—	+	–	—	+	–	—
13	+ +	+ +	1:10	–	–	—	–	–	—
14	+ +	+ +	1:20	+	–	—	–	–	—
*15	+ +	+ +	1:40	–	–	—	–	–	—
16	+ +	–	1:160	+ +	–	1:20	+ +	–	1:20
17	+ +	+ +	1:10	+	–	—	+ +	–	—
18	+ +	–	1:160	–	–	—	+	–	—
19	+ +	+	nd	–	–	—	–	–	—
20	+ +	+	—	–	–	—	–	–	—
21	+ +	+ +	1:80	+ +	+ +	1:80	+ +	+ +	1:20
*22	+ +	+ +	1:320	+ +	+	—	+ +	+ +	—
*23	+ +	+	1:640	+ +	+ +	1:20	+ +	+	1:10
24	+ +	+ +	1:80	+ +	–	—	+ +	–	1:20

*: patients who relapsed clinically.
nd: not done.
–: negative IF; +: weakly positive IF; + +: positive IF.

showed IgG and C_3 deposits. Three of five patients with positive C_3 relapsed as did five of 19 patients negative for C_3.

The IIF was negative in the first two groups of patients, in those who showed negative or weakly positive DIF. Among the 10 who showed positive DIF, seven showed a positive indirect test in a titre of 1:10–1:80. Four of the seven patients relapsed clinically compared with four of 17 patients negative on IIF. There was no significant change in the titre of the antibodies at the second test.

DISCUSSION

The prognosis and survival of pemphigus patients has changed dramatically following the introduction of systemic steroids.[7] Further improvement has been achieved with combined therapy of corticosteroids and immunosuppressive drugs such as azathioprine,[8] gold[9] and wi plasmapheresis.[10] The course of the disease remains variable and it is difficult to predict wi accuracy the probable fate of any particular patient. In view of the long term period of therapy which pemphigus patients require and the potential hazards of adverse reactions secondary therapy there remains much risk in their management. Hence the need for reliable criteria the reduction of the dosage and the eventual withdrawal of their drugs. When patients are in t active phase of the disease the main parameter for reducing the dose of drugs is the clini response. Though the titre of circulating antibodies may be helpful in evaluation of disea severity, in an individual patient it is not a reliable test.[4,5] It is not so sensitive and considera variations in the titre may be seen using different substrates.[11] When the patient is in clini remission the decision about the further reduction in the dose of drugs can be very difficul

In this phase one cannot use clinical criteria for further lowering the dosage, and the IIF tes much less useful than when the disease was active. Relying on the IIF may result in inadequ treatment. In the absence of such criteria the therapeutic decisions are basically empirical.

Up till now the use of DIF in pemphigus patients in a state of clinical remission has be limited. It has been usually considered as a diagnostic test without prognostic significance. Ju and Lever[2] reported that it remains positive often for many years after the disease has subsid On the other hand, Bhogal *et al.*[12] mentioned that 10 pemphigus patients, out of 15 who w free of lesions, showed negative DIF. In our study eight patients showed negative DIF and o one of them relapsed clinically in a follow-up of 20 months compared with seven of 16 patie with weak or strong positive DIF. The IIF was negative in 17 patients and positive in sev patients. Four patients out of the seven relapsed clinically, while four of 17 patients w negative IIF showed recurrence. No significant difference in the titre was observed between t two time points of the testing. It seems that in a state of remission there is only a part correlation between the DIF and the IIF. It would appear that the DIF test is more sensiti than IIF. Small amounts of IgG deposits are detected in the epidermis by the DIF, while t IIF technique failed to detect similar amounts of circulating antibodies. Positive immu fluorescent findings in a state of remission may have a significant prognostic value as an indica of an immunological activity of the disease and, therefore, treatment should be maintained. our experience, the intensity of DIF staining might be roughly graded into weakly positive a strongly positive fluorescence. This may be important in the follow-up of patients who are f of lesions. DIF findings may be indicative of either further regression or immin exacerbation. Repeated negative DIF is a possible marker for apparent cure of the disea However, in our study one patient developed new lesions after being off treatment for one ye Obviously, additional experience with a prolonged period of follow-up is needed to clarify t point.

Opinions are divided in the literature regarding the possible role of third component complement (C_3) in the pathogenesis of pemphigus. The ability of pemphigus antibodies to C_3 *in vitro*[13] and the increased cell detachment following addition of complement to epiderm cell cultures treated with pemphigus antibodies[14] support the concept that complement m play a role in the process of acantholysis. On the other hand, the fact that in vitro PV antibo induced acantholysis was achieved in complement-depleted culture media[15] may oppose t concept. Recently, Anhalt *et al.*[16] were able to show that complement activation is not essential mechanism in experimental pemphigus in mice, but it does enhance the developm of cutaneous lesions. Interestingly, three out of eight patients, who relapsed clinically, show C_3 deposits in the epidermis months before the clinical relapse. In our opinion, presence of would seem to be an additional marker for disease activity.

In conclusion, DIF staining is not only a reliable diagnostic test for pemphigus, but it also can be of prognostic significance for patients who are in clinical remission. It may serve as an indicator for immunological activity of the disease and be of help in the therapy. We consider that maintenance therapy should not be stopped until both the DIF and IIF tests become negative.

REFERENCES

1 Beutner EH, Lever WF, Witebsky E *et al.* Autoantibodies in pemphigus vulgaris. *J Am Med Assoc* 1965; **192:** 682–8.

2 Judd KP, Lever WF. Correlation of antibodies in skin and serum with disease severity in pemphigus. *Arch Dermatol* 1979; **115:** 428–32.

3 Weissman V, Feuerman EJ, Joshua H, Hazaz B. The correlation between the antibody titre in sera of patients with pemphigus vulgaris and their clinical state. *J Invest Dermatol* 1978; **71:** 107–9.

4 Creswell SN, Black MM, Bhogal B *et al.* Correlation of circulating intercellular antibody titres in pemphigus with disease activity. *Clin Exp Dermatol* 1981; **6:** 477–83.

5 Fitzpatrick RE, Newcomer VD. The correlation of disease activity and antibody titres in pemphigus. *Arch Dermatol* 1980; **116:** 285–90.

6 Beutner EH, Nisengard RJ, Kumar Y. Defined immunofluorescence basic concepts and their application to clinical immunodermatology. In. *Immunopathology of the Skin*, 2nd edn., London: John Wiley & Sons, 1979; 29.

7 Lever WF, White H. Treatment of pemphigus with corticosteroids: results obtained in 46 patients over a period of 11 years. *Arch Dermatol* 1963; **87:** 12–26.

8 Aberer W, Wolff-Schreiner EC, Stingl G, Wolff K. Azathioprine in the treatment of pemphigus vulgaris. *J Am Acad Dermatol* 1987; **16:** 527–31.

9 Penneys NS, Eaglstein WH, Frost P. Management of pemphigus with gold compounds. *Arch Dermatol* 1976; **112:** 185–7.

10 Auerbach R, Bystryn JC. Plasmapheresis and immunosuppressive therapy. *Arch Dermatol* 1979; **115:** 728–30.

11 Judd LP, Mescon H. Comparison of different epithelial substrates useful for indirect immunofluorescent testing of sera from patients with active pemphigus. *J Invest Dermatol* 1979; **72:** 314–16.

12 Bhogal B, Wojnarowska F, Black MM *et al.* The distribution of immunoglobulins and the C_3 component of complement in multiple biopsies from the uninvolved and perilesional skin in pemphigus. *Clin Exp Dermatol* 1986; **11:** 49–53.

13 Kawana S, Janson M, Jordan RE. Complement fixation by pemphigus antibody. I. *In vitro* fixation to organ and tissue culture skin. *J Invest Dermatol* 1984; **82:** 506–10.

14 Kawana S, Geogheyan WD, Jordan RE. Complement fixation by pemphigus antibody. II. Complement enhanced detachment of epidermal cells. *Clin Exp Immunol* 1985; **61:** 517–25.

15 Woo TY, Hogan VA, Patel H *et al.* Specificity and inhibition of the epidermal cell detachment induced by pemphigus IgG *in vitro*. *J Invest Dermatol* 1983; **81:** 115s–21s.

16 Anhalt GJ, Till GO, Diaz LA *et al.* Defining the role of complement in experimental pemphigus vulgaris in mice. *J Immunol* 1986; **137:** 2835–40.

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Allergic and irritant contact dermatitis compared in the treatment of alopecia totalis and universalis. A comparison of the value of topical diphencyprone and tretinoin gel

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SUMMARY

Diphencyprone is a potent topical sensitizer, but is non-mutagenic in the Ames test (unlike dinitroclorobenzene) and remains relatively stable in solution (unlike squaric acid dibutyl ester).

Seventeen patients with total loss of scalp hair (eight alopecia totalis, nine alopecia universalis) were treated by maintaining on one side of the scalp an allergic contact dermatitis induced by 2,3 diphenylcyclopropenone-1 ('diphencyprone'), and on the other side an irritant contact dermatitis using tretinoin gel (Retin A). After 20 weeks, treatment with tretinoin was stopped and diphencyprone was applied bilaterally for a further 10 weeks.

Satisfactory regrowth of terminal hair on the scalp was achieved in only one patient. Eyebrow, eyelash and beard regrowth was achieved in one individual whilst in another, moderate, but not cosmetically satisfactory, scalp regrowth took place. In no patient did regrowth take place at tretinoin treated sites until after diphencyprone was substituted.

The induction of both allergic and irritant forms of contact dermatitis have in the past been advocated for the treatment of alopecia, mainly alopecia areata,[1,2] although it has recently been suggested that dermatitis without an allergic component is ineffective.[3] The assessment of the effectiveness of different treatments in alopecia areata is, however, confused by the frequency of spontaneous remission in this condition.

We have, therefore, chosen to study only patients with alopecia totalis and alopecia universalis, both conditions in which spontaneous recurrence is a much rarer event. We have treated the scalps of eight patients with alopecia totalis and nine with alopecia universalis by maintaining, in each individual, on one side of the scalp an allergic contact dermatitis and on the

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other side an irritant contact dermatitis. In this way we were able to directly compare these two different types of treatment.

In most previous clinical studies either dinitroclorobenzene (DNCB) or squaric acid dibutylester (SAD) have been used as sensitizers. DNCB is no longer recommended for this purpose because of mutagenic potential.[4] SAD is unstable in solution and, therefore, difficult to handle in the clinical situation. As our sensitizer we chose 2,3 diphenylcyclopropenose-1 (usually shortened to 'diphencyprone') because this compound is non-mutagenic in the Ames test[5,6] and if stored protected from light remains stable for up to 4 weeks.[7] Tretinoin gel (Retin-A, Roche Products Ltd, Welwyn Garden City, U.K.) was used as the irritant.

PATIENTS

Ethical Committee approval and informed consent having been obtained, 17 volunteer patients with complete loss of scalp hair were studied. Of these, eight had loss mainly confined to the scalp (two with additional loss of eyebrows, eyelashes or beard) and the other nine had universal loss of body hair. The age range of the patients was from 5 years to 72 years, though only three were below 20 years. The youngest patient was treated only after extreme parental pressure, and a detailed discussion with the parents.

At the first visit all patients were examined and a detailed history of duration of hair loss was obtained together with a history of any preceding hair loss and rate of disease progression. In addition, particular attention was paid to obtaining an accurate personal and/or family history of hypertension, alopecia areata, autoimmune thyroid disease, vitiligo, diabetes and atopy—all of which are associated with alopecia areata and some of which are of prognostic importance. The extent and type of hair loss was noted and the presence of nail pitting or ridging was recorded.

A family history of alopecia areata was present in two patients, and four patients were atopic with a personal history of asthma, hay fever and/or atopic dermatitis, whilst a further three had a family history of atopic disorders. No patient was found to be hypertensive, though four gave a family history. In three patients there was a family history of thyroid disease. None of the subjects were themselves affected by or gave a family history of vitiligo. One patient had maturity onset diabetes, but there was no family history in this or any other patient.

METHODS

Diphencyprone (Aldrich Chemical Co., Gillingham, U.K.) was prepared in acetone and kept in tinted bottles in a dark cupboard when not in use. The entire batch of dilutions was changed for a fresh supply each month. We were thus able to ensure the application of active sensitizer at all times.[7]

Sensitization was induced by the application of 2% diphencyprone to one half of the scalp only. In sensitized individuals an active dermatitis was thereafter maintained by the weekly application of diphencyprone in concentrations varying from 0·0001% to 2%. The degree of dermatitis maintained was such that the inflammation induced had settled to a slightly scaly mild erythema by the time of their next visit for treatment, 7 days after application. Treatment was applied by using a cotton wool applicator to spread 0·5–1 ml of solution evenly over one half of the scalp on each occasion. This was carried out by medical personnel.

To the opposite side of the scalp the patients themselves applied tretinoin 0·25% gel (Retin A, Roche) once or twice daily as required in order to maintain a dermatitis of similar severity.

In this way each patient was treated with a contact dermatitis of an allergic type to one side of the scalp and of an irritant type to the other.

After 20 weeks all remaining participants stopped treatment with tretinoin and the whole scalp was thereafter treated with diphencyprone for the remainder of the trial (at least a further 10 weeks).

RESULTS

Of the 17 patients initially entering the study, 12 received a total of at least 30 weeks treatment.

In only one patient was initial sensitization not achieved and this patient was thereafter excluded from the study. The patient was a 28-year-old male with no personal or family history of alopecia, atopy, hypertension, vitiligo, thyroid disease or diabetes.

One patient withdrew after 3 months' treatment following a particularly severe dermatitis reaction to diphencyprone. At the time of withdrawal there had been no regrowth. Two patients withdrew because of difficulty attending for treatment on a weekly basis. In a further two patients, although initial sensitization was achieved satisfactorily, gradual loss of sensitivity followed even using the 5% concentration of diphencyprone. Despite this lost ability to react on the scalp, a marked dermatitis was produced in both patients on the volar aspect of an uninvolved forearm with only a 1% or 2% solution, but this was not followed by a return of scalp reactivity. One of these patients withdrew at 25 weeks and the other after the full 30 weeks.

Though some patients were able to maintain only a mild dermatitis on the tretinoin treated side, others were able to maintain active dermatitis, but none was able to promote even minimal hair growth. After 20 weeks of unsuccessful treatment with tretinoin this was stopped and diphencyprone was applied to both sides of the scalp thereafter.

At the diphencyprone treated sites most patients maintained an active dermatitis for a prolonged period of time. The concentration of diphencyprone required varied from 0·0001% to 2%.

Of the 12 patients who received a full 30 weeks treatment, three had shown a therapeutic response and treatment was further continued in these individuals. In the nine unsuccessfully treated patients all had maintained a satisfactory dermatitis (i.e. mild to moderate erythema and scale persisting for 7 days after application) but with no hair growth. These non-responders were then excluded.

Of the three patients who showed a response two had developed terminal hair growth at treated sites and a further one had developed growth at non-treated sites (eyebrows and eyelashes).

After 20 weeks treatment no patient had shown any response on the tretinoin treated side of the scalp. Diphencyprone was thereafter applied to both sides in all remaining patients.

Of the two patients with diphencyprone-induced scalp regrowth, one was exquisitely sensitive and required only a 0·0001% concentration to maintain a reaction, whilst the other was only moderately sensitive and required 1–2% to maintain a dermatitis.

The individual achieving the most striking improvement was a 25-year-old male who had suffered from total alopecia for 21 years, preceded by alopecia areata for 1 year before that. This individual had no personal or family history of hypertension, thyroid disease, vitiligo, diabetes or atopy, no family history of alopecia areata and had normal nails. Quite marked terminal hair growth was noted only on the diphencyprone treated side after 14 weeks and at 20 weeks there was a clear difference between the two sides. In addition, this patient had regrown both eyebrows—areas which had received no direct treatment. Following the application of diphencyprone, bilaterally widespread terminal hair growth was stimulated (Fig. 1).

In the other scalp responder, only moderate terminal hair regrowth was stimulated, but

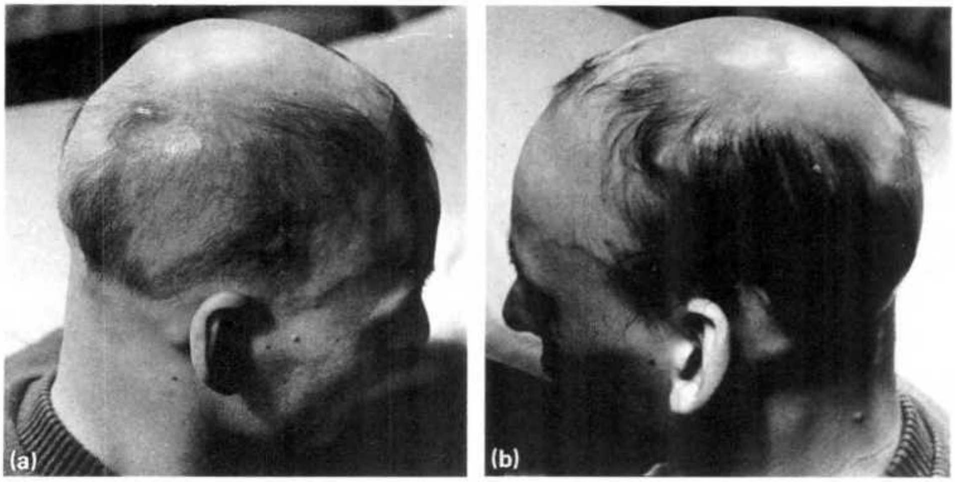

FIGURE 1. Diphencyprone induced regrowth at (a), week 15 and (b), week 25 (opposite side of scalp). Also note eyebrow regrowth and reactive post-auricular lymphadenopathy.

cosmetically satisfactory regrowth was not achieved. This individual was a 56-year-old man with a 38-year history of universal alopecia preceded by an additional 4 years with alopecia areata. His daughter had alopecia areata and he had nail pitting and ridging.

In the patient whose regrowth coincided with treatment, but was not in specifically treated areas (eyebrows, eyelashes and beard areas regrew) and where there was a negative personal/family history, total alopecia had been present for 56 years and he had longitudinal nail ridging on examination. This patient had noticed minimal thickening of the eyebrows prior to commencing treatment, during which he displayed very convincing improvement in terms of both hair density and area of growth, but it is possible that this might have been a continuing spontaneous regrowth. A concentration of 0·5–1% was required to maintain scalp dermatitis in this patient.

Of the non-responders, only one had no family or personal history of note though this 33-year-old female had previously become hypertensive whilst on the oral contraceptive.

Three of the adult non-responders and one responder had normal finger nails. Examination of the nails was, therefore, not of predictive value. Similarly, neither the duration of alopecia, nor the age at onset correlated with regrowth.

DISCUSSION

We have shown that the maintenance of a mild to moderate irritant contact dermatitis for 20 weeks is ineffective in the treatment of alopecia totalis and the scalp involvement of alopecia universalis. However, in the same individual the maintenance of an diphencyprone-induced allergic dermatitis is occasionally associated with some hair regrowth.

Diphencyprone is non-mutagenic in the Ames test[5,6] and if stored correctly, remains active and allows convenient clinical usage.[7] These are distinct advantages over the more commonly used topical sensitizers DNCB and squaric acid dibutyl ester. Diphencyprone has recently been used, mainly in the treatment of alopecia areata, with a higher response rate than we have found in the more severe problems of alopecia totalis and universalis.[8,9] However, alopecia areata differs from the more severe total and universal alopecias in that spontaneous regrowth is much commoner and alopecia totalis/universalis is associated with a higher incidence of autoantibodies and a poorer response to treatment with contact sensitizers.[10]

We have also shown that irritant contact dermatitis in our hands is ineffective in the treatment of total and universal alopecia. Incompletely understood immunological changes take place in the involved areas of hair loss and even the same individual may react vigorously to diphencyprone at one body site and not at another; as in our patients who lost their ability to react on the affected scalp, but could still mount a vigorous response to lower concentrations on unaffected forearm skin. A greater understanding of these changes would help rationalize our therapeutic approach to different treatments such as topical steroids, irritant/allergic contact dermatitis or UV irradiation and perhaps help to identify potential responders for a particular treatment modality.

There was no clear association in our study between age of patient, findings on initial examination, historical features, duration of disease, or strength of diphencyprone required to maintain dermatitis and hair growth. Our most notable success was achieved in an individual with alopecia totalis who had normal finger nails and no family history of hypertension, alopecia areata, atopy, vitiligo, diabetes or autoimmune thyroid disease.

Our experience of only occasional response to diphencyprone is in agreement with one previous study,[9] though another group have had a much higher success rate.[8] The reason for these differences is not clear though the duration of treatment and degree of dermatitis maintained may be of importance.

In conclusion, diphencyprone induced allergic contact dermatitis cannot be recommended as a routine treatment for total or universal alopecia.

REFERENCES

1 Schmoeckel C, Weissmann I, Plewig G. Braun-Falco O. Treatment of alopecia areata by anthralin-induced dermatitis. *Arch Dermatol* 1979; **115**: 1254–5.

2 Fiedler-Weiss VC, Buys CM. Evaluation of anthralin in the treatment of alopecia areata. *Arch Dermatol* 1987; **123**: 1491–3.

3 Swanson NA, Mitchell AJ, Leahy MS *et al.* Topical treatment of alopecia areata. Contact allergen versus primary irritant therapy. *Arch Dermatol* 1981; **117**: 384–7.

4 Strobel R, Rohrborn G. Mutagenic and cell transforming activities of 1-chlor, 2–4 dinitrochlorobenzene (DNCB) and squaric acid dibutylester (SADBE). *Arch Toxicol* 1980; **45**: 307–14.

5 Stute VJ, Hausen BM, Schulz KH. Diphenylcyclopropenonein stark wirksames Kontaktallergen. *Derm Beruf Umwelt* 1981; **29**: 12–15.

6 Wilkerson MG, Connor TH, Henkin J *et al.* Assessment of diphenycyclopropenone for photochemically induced mutagenicity in the Ames assay. *J Am Acad Dermatol* 1987; **17**: 607–11.

7 Wilkerson MG, Henkin J, Wilkin JK. Diphenylcyclopropenone: Examination for potential contaminants, mechanism of sensitisation and photochemical stability. *J Am Acad Dermatol* 1984; **11**: 802–7.

8 Happle R, Hausen BM, Wiesner-Menzel L. Diphencyprone in the treatment of alopecia areata. *Acta Dermato-venereol (Stockh)* 1983; **63**: 49–52.

9 Orecchia G, Rabbiosi G. Treatment of alopecia areata with diphencyprone. *Dermatologica* 1985; **171**: 193–6.

10 Friedmann PS. Alopecia areata and autoimmunity. *Br J Dermatol* 1981; **105**: 153–7.

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Delayed pressure urticaria, objective evaluation of a variable disease using a dermographometer and assessment of treatment using colchicine

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SUMMARY

A randomized double-blind placebo controlled trial of colchicine in the treatment of 13 patients with delayed pressure urticaria enabled us to assess some of the variables in this disorder. We have modified a previously described method of pressure testing using a calibrated dermographometer and shown a pressure induced papular dose response curve. Assessment of disease activity was based on the number of pressure weals which occurred, the size of delayed pressure induced papules using a dermographometer calibrated at $9{\cdot}75 \times 10^5$ pascals for five separate time periods on the back and estimations of erythrocyte sedimentation rate and the acute phase protein levels. We have been unable to show a therapeutic effect using colchicine 0·5 mg bd for 1 week.

In delayed pressure urticaria, delayed erythema and oedema occurs in association with marked subcutaneous swelling following pressure applied to the skin. This swelling occurs between 30 min and 9 h after application of pressure and may be present for up to 48 h.[1] An association with chronic urticaria occurs in over 90% of cases.[2] The disease is very variable in severity and in some the disability is considerable, particularly those with physically demanding occupations.

The histopathological feature of the early lesions of delayed pressure urticaria is an infiltrate composed mainly of neutrophils and eosinophils.[3] If the early neutrophil phase of inflammation could be inhibited the development of lesions might be controlled. Colchicine decreases polymorphonuclear leucocyte chemotaxis[4] and inhibits human monocyte unstimulated migration.[5] As there is no single effective treatment for delayed pressure urticaria, we have assessed colchicine as a treatment for this condition.

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METHODS

The diagnosis of delayed pressure urticaria (DPU) was based on a history of delayed swelling following application of pressure to the skin and the development of papular responses hours after pressure testing using a calibrated dermographometer. Male and female patients with active disease were included in the trial and all were out-patients of St. John's Hospital for Diseases of the Skin. Patients with significant medical illness were excluded, as were women capable of becoming pregnant, unless taking regular contraceptive precautions. Thirteen Caucasian patients (five males, eight females) aged between 28 and 54 years (mean 42 years) took part. Their DPU had been present between 1 and 17 years (mean 6 years). All had chronic urticaria, three had attacks of angioedema and one had mild cold urticaria. None were taking any drugs either for their DPU or for any medical condition, but three were taking antihistamines for chronic urticaria. No antihistamines were taken for at least 3 days prior to their first visit and none had taken long-acting antihistamines.

A randomized crossover double-blind trial of colchicine versus placebo was undertaken between July 1987 and January 1988. Colchicine was obtained from Evans Medical Limited, Langhurst, Horsham, England and an identical placebo from Penn Pharmaceutical Limited, Penn, Buckingham U.K. The trial was of 3 weeks' duration. During the 1st week either colchicine 0·5 mg or a placebo tablet were given twice daily. This dose of colchicine was chosen in order to minimize the possibility of gastrointestinal side effects. Patients were instructed to take a tablet first thing in the morning and again 12 h later. The 2nd week was a drug free period. During the 3rd week the alternative tablets were taken as during the 1st week.

Assessment of disease activity was based on patients' daily pressure weal counts, and the response to testing with a calibrated dermographometer. The patients filled in a diary card every night documenting the number of pressure weals that appeared during the previous 24 h, the sites at which they occurred and any side-effects from the tablets. These cards were collected at the end of each 7-day period. At each visit a dermographometer calibrated at $9{\cdot}75 \times 10^5$ pascals/sq mm was held firmly at right angles to the back, lateral to the spine for 5, 15, 33, 70 and 100 s. Pressure applications were approximately 5 cm apart. Each successive application was more caudal so that the final 100 s application was applied at the level of the lower thoracic spine (Fig. 1). By alternating from right to left of spine at each visit a total of two readings to the right and two to the left were obtained. All pressure applications and all measurements were done while the patients were prone and under the same conditions by the same observer. The two largest diameters at right angles of the pressure papules produced at each point were measured 6 h later in all patients. These measurements were later expressed as a mean of both diameters.

Four independent measurements of weal diameter were available on each patient at each pressure application. Results for each pressure application will be presented separately. For each patient, the difference between the pre-treatment and post-first treatment (week 1) weal diameter was compared with the difference between the treatment free (week 2) and post-second treatment (week 3) diameter. Thus for each patient the two differences represented the effect on weal diameter of the two drugs. These were then compared using standard analysis for the two period crossover trial,[6] which involves estimating the treatment effect, the period effect (whether a different effect is produced by the drug given first than the drug given second, regardless of which drug it is), and the treatment/period interaction (whether the effect of colchicine compared to that of placebo varied according to whether it was given first or second).

Assuming no true effect of treatment or period, it was possible to take the four measurements and calculate within subject variation.

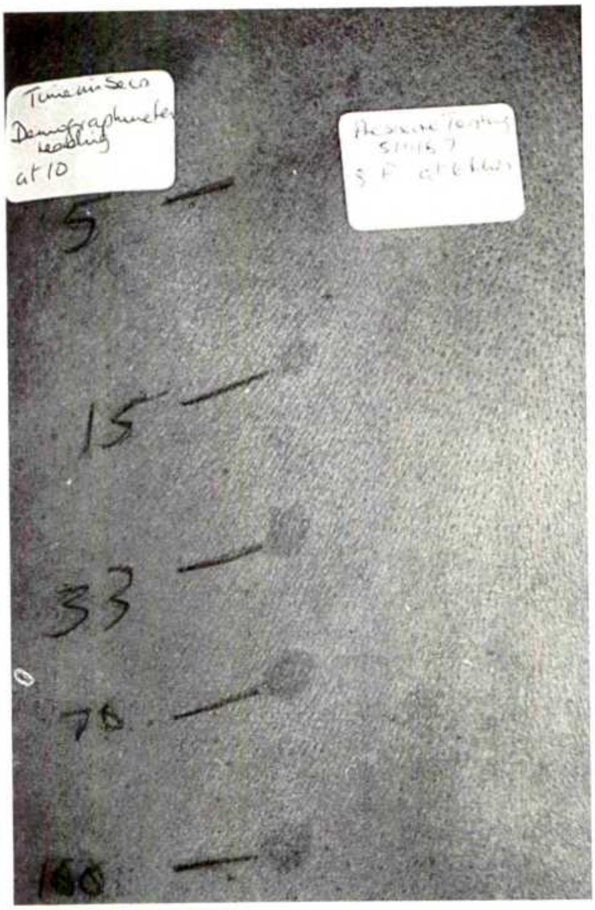

FIGURE 1. Dermographometer induced delayed pressure papules at 6 h.

At each visit full blood count including a total white cell count and differential and the erythrocyte sedimentation rate were performed. Also 15 ml of venous blood was obtained from each patient for acute phase protein estimation. Five millilitres were anticoagulated with potassium EDTA (ethylene diamine tetraacetic acid) and 10 ml were allowed to clot at 37°C. Both were centrifuged within 2 h and plasma and serum stored at 4°C prior to analysis. Serum levels of alpha 1 antitrypsin, antichymotrypsin, C^1 esterase inhibitor and C reactive protein were assayed by single radial immunodiffusion. Plasma levels of antithrombin III were measured by single radial immunodiffusion and complement components C_3 and C_4 by immunoturbidimetry. C reactive protein was estimated in eight patients.

RESULTS

All 13 patients completed the trial. One went into complete spontaneous remission after her first visit so results are given for 12 patients.

Five reported subjective improvement with colchicine compared with the placebo. One reported subjective improvement with placebo compared to colchicine and six found little difference between colchicine and placebo. No major side effects were noted. The diary card weal counts correlated well with patients' impressions. When the total number of weals present during the week colchicine was taken were compared with the total number present during the week placebo was taken, more weals were found to be present during the week colchicine was taken. The mean difference between these weeks showed an increase of 2·75 weals during the week colchicine was taken and the standard deviation of differences was 28·18 ($P > 0{\cdot}2$, paired t-test). These data do not show that colchicine affects weal counts in any way.

Mean diameters of all dermographometer induced pressure papules are shown in Figure 2 and mean change in pressure induced papule diameter for different pressure applications, by treatment and period, are shown in Table I. There was no evidence of any difference in the effect of colchicine compared to placebo whether or not colchicine was administered first or second. There was no significant beneficial effect of placebo. There was no overall mean difference between changes effected by colchicine as against placebo when estimated allowing for any period effect.

Because there was no clear evidence for any effect of either treatment or period we calculated the within subject variability in pressure induced papules over the four visits. We found the within subject standard deviations to be 1·49, 1·72, 2·62, 3·10 and 4·25 mm for the 5, 12, 33, 70 and 100 s pressure applications, respectively. These indicate the spread of individual values for a given subject around that subject's mean. Between subject standard deviations, also estimated[7] were 3·00, 3·34, 2·96, 3·22 and 3·41 mm, respectively. These represent the spread of individual subjects' mean values around the overall mean. The percentage of variance demonstrated by

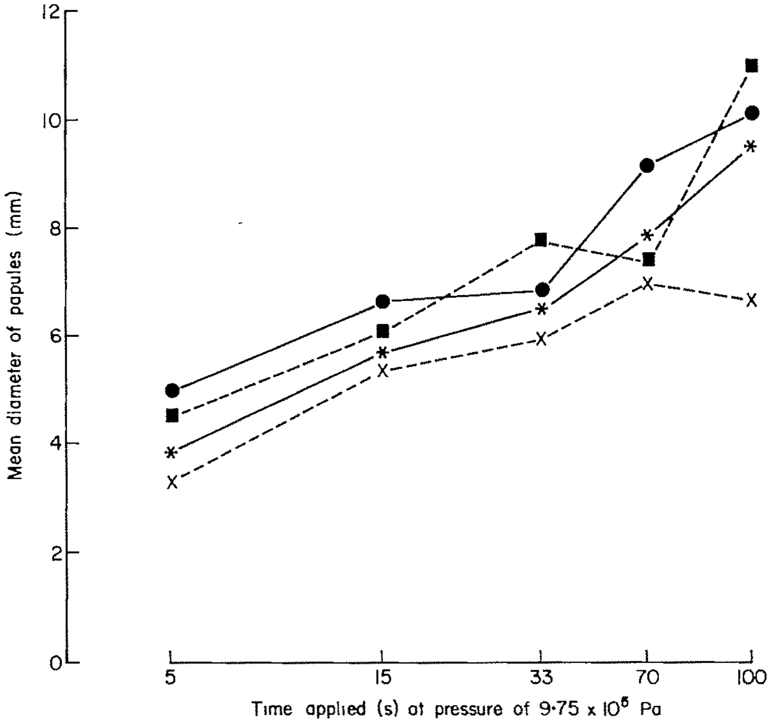

FIGURE 2. The mean papule diameter for all patients is shown in millimetres at each time point for colchicine, placebo and no treatment. ■ initial visit; X placebo; ● week without treatment; * colchicine.

TABLE 1. Mean change in diameter of all pressure induced papules during treatment for different pressure applications, by treatment and period (standard deviation in parentheses).

	Colchicine	Placebo
Period 1 (s)		
5	−0·10 (0·54)	0·50 (12·10)
15	0·70 (1·26)	−0·21 (1·87)
33	0·25 (1·03)	0·63 (4·35)
70	−0·55 (3·42)	1·00 (7·22)
100	−2·35 (4·35)	6·07 (9·34)
Period 2 (s)		
5	1·93 (2·96)	1·60 (3·05)
15	1·57 (2·73)	1·45 (1·28)
33	0·14 (3·29)	2·13 (2·14)
70	2·00 (3·04)	3·20 (2·02)
100	1·71 (3·55)	3·20 (3·71)

If the mean diameter was increased after treatment with either colchicine or placebo, this is shown by a negative sign.

within subject variability can be expressed as 25%, 21%, 44%, 48% and 61% for the five pressure applications. It can be seen that apart from the two lowest pressure applications the diameter of the pressure induced papules produced by the same pressure in each patient vary from week to week as much as the diameters of pressure induced papules produced by the same pressures vary from patient to patient.

Erythrocyte sedimentation rate (ESR) estimations done on 39 blood samples over the four visits during the three week period showed seven estimations (17·9%) to be raised (normal up to 10 mm/h). The abnormal values ranged between 16 mm/h and 90 mm/h with a mean of 30 mm/h and did not correlate with active treatment. Total white cell counts in 41 samples showed four (9·7%) to be raised (normal 4–11 × 10^9/l). These patients showed a neutrophil leukocytosis without any eosinophilia and the highest white cell count was 13·4 × 10^9/l. There was a modest elevation of ACT in the pretreatment samples of three patients (25%) but no other consistent changes and these elevations were not repeated.

DISCUSSION

We have not demonstrated a therapeutic effect of colchicine in delayed pressure urticaria. With the small numbers of subjects studied only a major effect would have been likely to have been detected. All patients had co-existing chronic urticaria which it is our impression occurs in the majority with DPU.

Clinical improvement during this study was assessed on the basis of weal counts over each 24-h period. Patients were instructed to count larger pressure weals only, but found it difficult to distinguish between pressure induced lesions from those of chronic urticaria. The subjective assessment of clinical improvement or worsening refers to the combined activity of their chronic urticaria and delayed pressure urticaria.

The method of pressure testing used is based on that described by Estes and Yung,[8] who found the threshold response to pressure testing to be related to the amount of pressure, duration of application and anatomic area tested. Our pressure of $9{\cdot}75 \times 10^5$ pascals equals 99·4 g/mm^2 ($1\text{Pa} = 0{\cdot}0197 \times 10^{-4}$ g/mm^2). The pressure/time applications are based on our previous experience and were designed to produce a graded response in terms of increasing papule size and an increase in the number of positive responses from 5 s to 100 s. The method is sensitive and reproducible. Papular responses increased in size from 5 s to 100 s (Fig. 2). The percentage of positive pressure tests produced are as follows: 51% positive at 5 s, 81% positive at 15 s, 86% positive at 33 s, 91% positive at 70 s and 93% positive at 100 s. The erythrocyte sedimentation rates and acute phase protein levels did not show consistent elevation and were not affected by any treatment given. White cell counts were not lowered by the dose of colchicine given during the trial.

As analysis focused on within subject changes, we have been able to document within subject variability. The within subject standard deviations are substantial, sometimes exceeding one third of the observed mean value (depending on pressure/time applications). In a condition where there is such a large within subject variability an extremely effective drug is needed to show a therapeutic effect using this method.

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We are grateful to Mrs June Isaacs for her skilful assistance in this trial.

REFERENCES

1 Dover JS, Kobza Black A, Milford Ward A, Greaves MW. Delayed pressure urticaria. Clinical features, laboratory investigations and response to therapy. *J Am Acad Dermatol* 1988; **18** (6): 1288–98.

2 Sussman GL, Harvey RP, Schocket AL. Delayed pressure urticaria. *J All Clin Immunol* 1982; **70**: 337–42.

3 Winkelmann RK, Black AK, Dover J, Greaves MW. Pressure urticaria—histopathological study. *Clin Exp Dermatol* 1986; **11**: 139–47.

4 Ehrenfeld M, Levy M, Barkli M *et al.* Effect of colchicine on polymorphonuclear leucocyte chemotaxis in human volunteers. *Br J Clin Pharm* 1980; **10**: 297–300.

5 Zakhireh B, Malech HL. The effect of colchicine and vinblastine on the chemotactic response of human monocytes. *J Immunol* 1980; **125** (5): 2143–53.

6 Armitage P, Berry G. *Statistical Methods in Medical Research.* 2nd edition, Oxford. Blackwell, 1987; 222–6.

7 Armitage P, Berry G. *Statistical Methods in Medical Research.* 2nd edition, Oxford: Blackwell, 1987; 196–8.

8 Estes SA, Yung CW. Delayed pressure urticaria: an investigations of some parameters of lesion induction. *J Am Acad Dermatol* 1981; **1**: 25–31.

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Urticaria successfully treated by desensitization with grass pollen extract

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SUMMARY

We report a case of severe incapacitating seasonal urticaria and angioedema in an 11-year-old boy due to grass pollen sensitivity, who responded well to desensitization therapy with Timothy grass pollen extract.

The use of desensitization therapy was first reported for hay fever in 1911.[1] Desensitization has been used recently for asthma, rhinitis, insect sting anaphylaxis, atopic dermatitis and chronic urticaria including the physical urticarias.[2–7]

Classical allergy is thought to occur in only a minor proportion of patients with urticaria attending hospital dermatology clinics.[8] However, Numata *et al.* showed that 30 out of 52 chronic urticaria patients had positive skin tests to house dust mite antigen.[9] No data on the effect of avoidance and challenge with house dust were presented so that it is not clear how often this is a cause for urticaria.

Seasonal urticaria due to pollen is infrequent and there are few reports of successful treatment by specific desensitization.[10] Such cases are usually due to food, drugs or physical agents. We report a case of a patient with severe seasonal urticaria and angioedema who responded to a course of desensitization with Timothy grass pollen extract.

CASE REPORT

An 11-year-old Caucasian boy was first seen in August 1985, and from the age of 3 had severe recurrent episodes of generalized urticaria and angioedema which included swelling of the throat. These occurred each year between May and August with less troublesome episodes in early April and September. The urticaria was minimal in winter. He also suffered from asthma in the summer months. He had a strong family history of atopy but none of angioedema. The strongly seasonal symptomatology suggested allergy to a pollen.

Investigations showed an elevated serum IgE level at 300 iu/ml and RAST + + + + to both Timothy grass and mixed grasses with negative RAST to cat, house dust and mould. The C1 esterase inhibitor levels, full blood count and differential white count were all normal.

Treatment with high doses of terfenadine had been of some benefit, but the urticaria and angioedema became more severe with considerable loss of time from school and necessitated from 1982, continuous oral steroid therapy between April and September.

DESENSITIZATION TREATMENT

Immunotherapy with grass pollen extract was started in January 1987 with a conventional schedule of weekly subcutaneous injections of Spectralgen®. This is freeze-dried Timothy grass pollen extract suspended in a diluent consisting of human serum albumen and water for injection preserved with phenol. The schedule began with a dose of 20 Biological Units and was increased according to the manufacturers schedule[11] unless the local reactions were more than 10 cm in diameter. These were controlled with indomethacin 25 mg t.d.s. on the day of injection and for the following 2 days.[11]

The maintenance dose of 100,000 units was reached after 6 months in June 1987 and this was continued every 4 weeks for 1 year whereupon the intervals were increased to 6–8 weeks. Since desensitization he has had two brief episodes of urticaria at the height of the pollen count in June 1988[12] when cycling along country lanes for several hours.

Asthmatic episodes are milder and less frequent than previously. There has been no change in the Class 4 RAST to Timothy and mixed grasses but IgE levels have declined slightly to 210 iu/ml.

DISCUSSION

Urticaria may be triggered by antigens absorbed percutaneously, nasally or both. Our patient observed that lying on grass produced immediate local lesions on the skin, as did the injections of the grass pollen antigen in the desensitizing vaccine. He would develop urticaria while indoors when there was no direct contact with pollen.

The seasonal exacerbation of this patient's symptoms led to the diagnosis of pollen-induced urticaria that was confirmed by a strongly positive RAST to Timothy grass.

When symptoms are disabling, the allergic cause of the urticaria has been shown and a specific vaccine is available for desensitization, this treatment may be invaluable as was found in our case.

REFERENCES

1 Noon L. Prophylactic inoculation for hay fever. *Lancet* 1911; **1:** 572–3.

2 Frankland AW, Augustin R. Prophylaxis of summer hay fever and asthma. *Lancet* 1954; **1:** 1055–7.

3 Aas K. Hyposensitization in house dust asthma. *Acta Paediat Scand* 1971; **60:** 264–8.

4 Hunt KJ, Valentine MD, Sabatzka Ak *et al.* A controlled trial of immunotherapy in insect hypersensitivity. *New Engl J Med* 1978; **209:** 157–61.

5 Strauss JS, Kligman AM. The relationship of atopic allergy and dermatitis. *Arch Dermatol* 1957; **75:** 806–11.

6 Leigh IM, Ramsey CA, Calnan CD. Cold urticaria "desensitization". *Trans St Johns Hosp Derm Soc* 1974; **60:** 40–2.

7 Kyellman B, Petterson R. Atopic disease and paediatric ambulatory care. *Allergy* 1980; **35:** 531–5.

8 Champion RH, Lachmann PJ. Specific desensitization in dermatology. In: *Recent advances in Dermatology* 7 (Champion RH, Ed) Churchill Livingstone Publications, 1986; 233–50.

9 Numata T, Yamamoto S and Yamura T. The role of mite antigen in chronic urticaria. *Annals of Allergy* 1979; **43:** 356–8.

10 Mathews KP. A current view of urticaria. *Med Clin North Am* 1974; **58;** 185–205.

11 Spectralgen®. *Pollens Information Sheet.* Pharmacia Diagnostic, Uppsala, Sweden.

12 Young R. Aboriginal promise of a long, sneezing summer. *The Times*, 21 June 1988.

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Treatment of actinic prurigo with PUVA: mechanism of action

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SUMMARY

Five patients with actinic prurigo were treated twice weekly with PUVA. One area on the back was shielded from UVA throughout the 15-week treatment period. Before PUVA, all patients had increased erythemal sensitivity to UVA and showed abnormal augmentation of UVB erythema by topical indomethacin. After PUVA, all patients were free of photosensitive symptoms and skin that had been exposed to UVA showed normal erythemal responses. By contrast, the areas of skin that had been protected from UVA showed erythemal responses that were unchanged from pre-PUVA values. Augmentation of UVB erythema by topical indomethacin persisted, both on UVA exposed and UVA protected skin.

These results show that, although PUVA is an effective treatment in actinic prurigo, it does not alter the underlying mechanism of photosensitivity. The protective effect is local and is due presumably to an increase in melanin pigmentation and epidermal thickness.

Actinic prurigo is an uncommon, chronic photosensitivity disorder that affects mainly females and can usually be distinguished clinically from polymorphic light eruption (PLE).[1] The relationship of actinic prurigo (as seen in Caucasians) to the identically named, often familial, photosensitivity disorder reported in North American Indians and Latin American subjects[2,3] is not clear. Action spectrum studies have shown that most patients with actinic prurigo have increased erythemal sensitivity to ultraviolet radiation (UVR), particularly UVA.[4] The mechanism of the abnormal response to UVR is not known but a recent study[5] has shown that UVB and UVA erythema are augmented by topical application of indomethacin in patients with actinic prurigo. This is in contrast to the inhibitory effect of indomethacin on UVB erythema that is seen in normal skin,[6] and suggests that lipoxygenase products of arachidonic acid may be involved in the mechanism of photosensitivity.[5]

Actinic prurigo is difficult to treat and topical sunscreens are of little help unless the cosmetically less acceptable reflectant ones are used.[1] Thalidomide is effective in the majority of patients[7,8] but use of this drug is limited by its well-known side-effects. Photochemotherapy

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with psoralen and UVA (PUVA) is an effective treatment for PLE[9,10] although its mechanism of action, whether immunological, pharmacological or merely photoprotective, is not known.

We now report the effect of PUVA in five patients with actinic prurigo. The previously described abnormality of response to indomethacin in this disease[5] has allowed the mechanism of action of PUVA to be studied.

METHODS

Patients

Five females (aged 30–39) years) with actinic prurigo were studied. All were photosensitive since early childhood and developed within hours of minimal sun exposure, patchy, raised areas of erythema. Excoriated papules and areas of lichenification were present, principally on the face, hands and other exposed sites. Although the skin changes were most marked during the summer months, the symptoms continued throughout the year. Unlike many patients with polymorphic light eruption, none of the patients that we studied developed increasing tolerance to sunlight during the later months of summer. Examination of urine, blood and faeces for porphyrins was normal in each case and none of the patients were taking any medication known to cause photosensitivity.

PUVA treatment

All patients were treated twice weekly for approximately 15 weeks with PUVA using 8-methoxypsoralen given orally (0·5 mg/kg) 2 h before UVA exposure. UVA doses of 0·25–0·5 J/cm^2 were used at the start of treatment and increased at weekly intervals to a maximum of 2–6 J/cm^2 (mean cumulative UVA dose 58 J/cm^2). Throughout the treatment period one area on the back of each patient (Fig. 1) was protected from UVA exposure using two layers of thick, tightly woven cotton sheeting shown to have negligible ($<0{\cdot}1\%$) UVA transmission.

Action spectrum studies

An irradiation monochromator[11] was used to give a series of increasing exposures (using a dose increment factor of $\sqrt{2}$) to the clinically uninvolved skin of the back at wavelengths of 300, 320, 340, 360, 380 and 400 nm. The irradiated sites were examined 24 h after exposure and the lowest dose of radiation that resulted in just perceptible erythema was taken as the minimal erythema dose (MED). In each patient the MED was determined before PUVA and after 15 weeks of treatment, both on areas of skin that had been exposed to UVA and areas that had been protected from UVA. The MED measurements were repeated on UVA exposed skin approximately 6 months after the last PUVA treatment.

Response of UVB erythema to indomethacin

The UVB source was a 500 W medium pressure mercury arc lamp in conjunction with Schott WG305 (3 mm thick) and UG5 (1 mm thick) colour glass filters. Approximately 97% of the erythemally effective power from this source lies within the UVB (290–320 nm) spectral interval.[12] Before the first PUVA treatment, the lamp was used to irradiate consecutively on the back of each patient two series of five circular sites (10 mm diameter). A dose increment factor of 1·3 was used and, in each case, the lowest dose was based on the previously determined MED at 300 nm for that patient. 1% indomethacin gel (100 μg/cm^2) was applied immediately after irradiation to one series of sites and an equivalent volume of the gel base was applied to the other series. All treated areas were then occluded under polythene for 2 h.

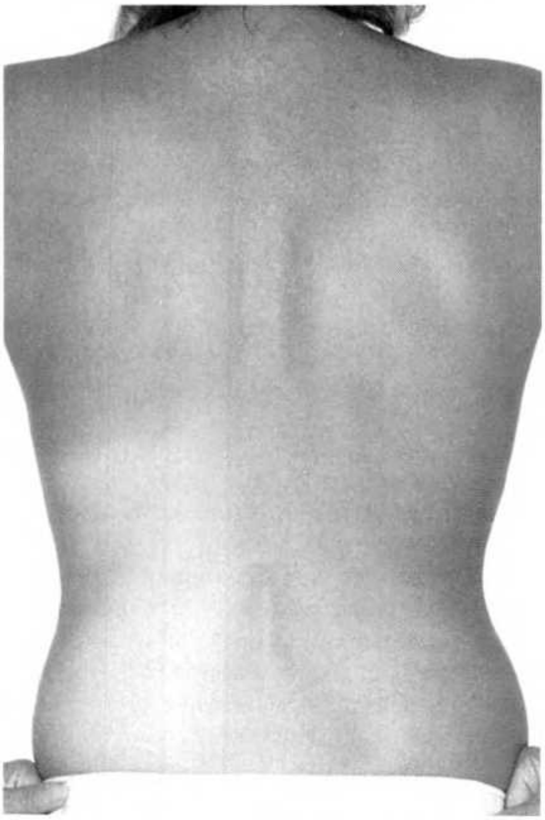

FIGURE 1. The back of one patient after treatment with PUVA showing the area protected from UVA exposure.

The intensity of erythema was measured using a reflectance instrument which compares the amount of red and green light reflected from the skin and thus obtains an 'erythema index' related to the blood content of the superficial dermis.[13] Before irradiation, three measurements of the erythema index were made at each of the sites with the subject lying prone on a couch. The measurements were repeated 24 h after irradiation and the increase in erythema index (ΔE) calculated for each site.[14]

The irradiation procedure, method of application of indomethacin and measurements of erythema were repeated after the 15-week course of PUVA treatment, both on areas of the back exposed to UVA and on adjacent areas that had been protected from UVA.

RESULTS

PUVA treatment

Apart from occasional episodes of mild erythema, the treatment with PUVA was uneventful. Four patients developed moderate pigmentation of the UVA-exposed skin and the remaining patient (number 2) showed only minimal pigmentation. Increased tolerance to sun exposure was reported after approximately 4 weeks of treatment and, at the end of the course of PUVA, all patients remained free of any photosensitive symptoms and rash despite long periods of summer sun exposure.

Action spectrum studies

The MEDs determined for each patient before and after the course of PUVA are shown in Table 1. Before PUVA, two patients showed increased erythemal sensitivity to UVB (300 nm) and all patients showed increased sensitivity within the UVA spectral interval (320–400 nm).

After PUVA treatment the MEDs determined on UVA protected skin showed little or no change from pre-PUVA values, whereas in each patient the MEDs determined on UVA exposed skin were within the range for normal skin at all of the wavelengths used (Table 1).

The MEDs determined on UVA exposed skin 6 months after the last PUVA treatment showed that in all patients the protection afforded by PUVA therapy, although still present, was less pronounced than immediately after the treatment course.

Response of UVB erythema to indomethacin

The measured increase in erythema index at the sites treated with indomethacin and at the sites treated with gel base was plotted against the logarithm of the UVB dose to obtain three pairs of dose–response curves in each patient: pre-PUVA; post-PUVA on treated skin; post-PUVA on UVA protected skin (Fig. 2). Linear regression was applied to the straight-line part of the curve to obtain a slope for each response, together with a 'threshold dose' defined[15] as that dose of UVB where the extrapolated linear regression line intersects the abscissa at $\Delta E = 0$. In each patient unequivocal augmentation of erythema was seen at the sites treated with indomethacin compared with the gel base treated sites. The dose–response curves for the gel base treated sites showed a parallel shift to the right on PUVA treated skin compared with the pre-treatment curves (Fig. 2).

In order to compare quantitatively any difference between the paired dose–responses (indomethacin and gel base treatment) in each patient, the dose of UVB required to produce a ΔE of 0·1 (approximately 2 MED, or erythema of moderate intensity) was determined from the slope of the dose–response curve and the threshold dose for the gel base treated sites. The degree of erythema (termed 'UVB indomethacin index') that would have resulted from exposure to this dose of UVB was then calculated for the sites treated with indomethacin. The UVB indomethacin index can be defined as

$$m_i[0{\cdot}1/m_g + \log(t_g/t_i)] \qquad (1)$$

where m_i and m_g are the slopes of the dose–response curves on the indomethacin and gel base treated sites, respectively; and t_i and t_g are the corresponding threshold doses. Values of the UVB indomethacin index $< 0{\cdot}1$ indicate inhibition of erythema by indomethacin (as seen in normal skin) and values $> 0{\cdot}1$ indicate augmentation.

The UVB indomethacin indices calculated for each patient are shown in Table 2. Before PUVA, all patients showed augmentation of UVB erythema by indomethacin. This abnormal response persisted after the course of PUVA, both on UVA exposed and UVA protected skin (Table 2). There was no significant difference between the UVB indomethacin indices determined pre-PUVA and those determined post-PUVA, either on UVA exposed or UVA protected skin ($P > 0{\cdot}1$, paired t-test).

DISCUSSION

All the patients studied had long-standing photosensitivity characterized by the development,

TABLE 1. Minimal erythema doses (MED) before and after PUVA therapy in different spectral wavebands

	MED (J/cm²)					
Subject	300 ± 5 nm	320 ± 10 nm	340 ± 20 nm	360 ± 20 nm	380 ± 20 nm	400 ± 30 nm
1 Pre-PUVA	0·001	0·05	1.7	5	14	28
7 days post-PUVA:						
protected skin	0·001	0·05	1·7	5	10	40
exposed skin	0·02	2	7	20	40	80
6 months post-PUVA:						
exposed skin	< 0·0005	0·08	0·9	2·5	10	14
2 Pre-PUVA	0·010	0·5	5	7	—	40
7 days post-PUVA:						
protected skin	0·028	1·4	5	10	—	40
exposed skin	0·056	2	14	20	—	40
6 months post-PUVA:						
exposed skin	0·020	1·4	10	14	20	28
3 Pre-PUVA	0·014	0·5	3·5	7	7	10
7 days post-PUVA:						
protected skin	0·014	0·5	3·5	5	7	10
exposed skin	0·080	5·6	28	> 40	> 60	> 80
6 months post-PUVA:						
exposed skin	0·056	—	10	—	—	—
4 Pre-PUVA	0·014	0·25	3·5	5	20	25
7 days post-PUVA:						
protected skin	0·014	0·25	3·5	10	20	25
exposed skin	0·080	5·6	28	56	> 40	> 50
6 months post-PUVA						
exposed skin	0·028	> 0·7	10	> 10	> 40	> 50
5 Pre-PUVA	0·056	1·4	3	3	10	> 14
7 days post-PUVA:						
protected skin	0·056	1·4	3	3	10	> 14
exposed skin	> 0·112	> 4	> 20	20	40	50
6 months post-PUVA:						
exposed skin	0·080	2	10	10	10	20
Normal range (95% confidence interval)	0·014–0·056	1–4	6–24	18–72	25–100	40–160

following minimal sun exposure, of patchy, palpable erythema on a background of chronic eczematous changes that persisted throughout the year. These clinical findings, together with the history of onset of photosensitivity in early childhood, and increased UVA erythemal sensitivity demonstrated by monochromator phototesting, indicate[1] a diagnosis of actinic prurigo rather than PLE.

The degree of photosensitivity was severe in all the patients and resulted in considerable

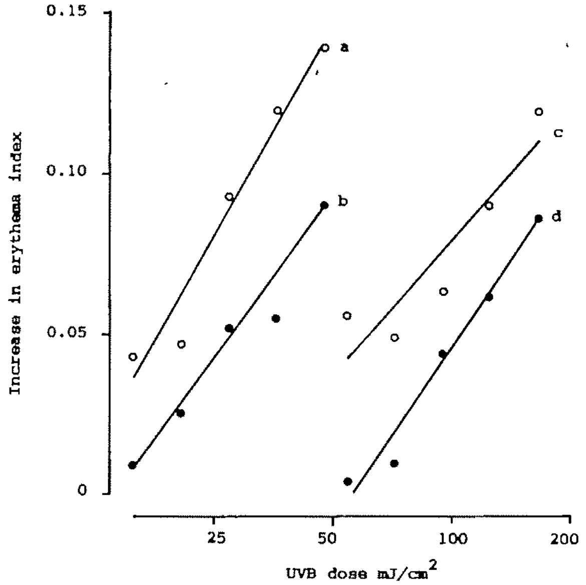

FIGURE 2 Dose–response curves in one patient (number 4) before PUVA (curves a and b) and after PUVA on UVA exposed skin (curves c and d). The open circles refer to sites treated with indomethacin and the closed circle refer to sites treated with gel base. The error bars have been omitted for clarity.

disruption of their lifestyle. In the past, sunscreens, topical and systemic corticosteroids, beta-carotene and hydroxychloroquine had been given with little or no benefit. Treatment with PUVA was well-tolerated by all of the patients. In each case, the starting dose of UVA that we used (0·25–0·5 J/cm^2) was considerably lower than the UVA MED (3–7 J/cm^2 at 360 nm). Occasional episodes of erythema occurred but these resolved without treatment and did not progress to form an eruption similar to that precipitated by sunlight. The patients all noticed improvement in their tolerance to sunlight within about 4 weeks of starting treatment and, by the end of the 15 weeks of treatment, all were able to tolerate long periods of intense sun exposure (both at home and abroad in some cases) with no symptoms. In keeping with this, the previously abnormal erythemal responses were found to be normalized on skin treated with

TABLE 2. Calculated UVB indomethacin indices before and after PUVA therapy

	UVB indomethacin index		
Patient	pre-PUVA	post-PUVA (protected skin)	post-PUVA (treated skin)
1	0·25	0·23	0·27
2	0·14	0·11	0·15
3	0·15	0·21	0·22
4	0·16	0·12	0·12
5	0·15	0·16	0·16

PUVA. Treatment with PUVA was stopped at the end of the summer. Six months later repeat monochromator phototesting showed that some degree of protection persisted, but return of clinical photosensitivity at about that time required treatment to be restarted.

In broad terms, a sun protection factor of about eight was achieved immediately following the course of PUVA treatment. This value is comparable with protection factors in PUVA treated normal skin that have been published by other workers,[16] but is appreciably greater than the protection factor of two to four normally found in unsensitized skin exposed to UVA.[17] It is clear that the protection achieved in our patients was due more to the epidermal thickening that is known to be provoked in PUVA treated skin,[18] than to the development of pigmentation. In keeping with this, one patient (number 2) developed only a minimal tan in response to PUVA and yet showed good protection as demonstrated both by monochromator testing (Table 1) and response to sun exposure.

Although PUVA has been used to treat several other photosensitivity disorders such as PLE,[9,10] solar urticaria[19] and chronic actinic dermatitis,[20] its mechanism of action is not known. Apart from stimulation of melanogenesis and epidermal thickening,[18] PUVA has numerous actions on human skin including local (and systemic) immunological effects[21,22] and enzyme inactivation.[23] The finding that the MEDs were unchanged on UVA protected skin (Table 1) shows that PUVA is acting only locally in actinic prurigo. That the abnormal response of UVB erythema to topical indomethacin was not altered by PUVA (Table 2), even on UVA exposed skin that now reacted normally to UVR in terms of the MED (Table 1), suggests that PUVA has not affected the underlying mechanism of photosensitivity. It seems most likely, therefore, that the effect of PUVA in actinic prurigo (and perhaps in other photosensitivity disorders) is purely photoprotective due to the local stimulation of melanogenesis and epidermal thickening.

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REFERENCES

1 Frain-Bell W. *Cutaneous Photobiology*. Oxford. Oxford University Press, 1985.
2 Epstein JH. Polymorphous light eruption. *J Am Acad Dermatol* 1980; **3:** 329–43.
3 Scheen SR, Connolly SM, Dicken CH. Actinic prurigo. *J Am Acad Dermatol* 1981; **5:** 183–90.
4 Addo HA, Frain-Bell W. Actinic prurigo—a specific photodermatosis. *Photodermatology* 1984; **1:** 119–28
5 Farr PM, Diffey BL. Augmentation of ultraviolet erythema by indomethacin in actinic prurigo: evidence of mechanism of photosensitivity. *Photochem Photobiol* 1988; **47:** 413–7.
6 Farr PM, Diffey BL. A quantitative study of the effect of topical indomethacin on cutaneous erythema induced by UVB and UVC radiation. *Br J Dermatol* 1986; **115:** 453–66
7 Calnan CD, Meara RH. Actinic prurigo (Hutchinson's summer prurigo). *Clin Exp Dermatol* 1977; **2:** 365–72.
8 Lovell CR, Hawk JLM, Calnan CD, Magnus IA. Thalidomide in actinic prurigo. *Br J Dermatol* 1983; **108:** 467–71.
9 Gschnait F, Honigsmann H, Brenner W *et al.* Induction of UV light tolerance by PUVA in patients with polymorphous light eruption. *Br J Dermatol* 1978; **99:** 293–5.
10 Parrish JA, Levine MJ, Morison WL *et al.* Comparison of PUVA and beta-carotene in the treatment of polymorphous light eruption. *Br J Dermatol* 1979; **100:** 187–91.
11 Diffey BL, Farr PM, Ive FA. The establishment and clinical value of a dermatological photobiology service in a district general hospital. *Br J Dermatol* 1984; **110:** 187–94.
12 Farr PM, Diffey BL, Humphreys F. A quantitative study of the effect of terfenadine on cutaneous erythema induced by UVB and UVC radiation. *J Invest Dermatol* 1986; **87:** 771–4.

13 Diffey BL, Oliver RJ, Farr PM. A portable instrument for quantifying erythema induced by ultraviolet radiation. *Br J Dermatol* 1984; **111:** 663–72.

14 Farr PM, Diffey BL. Quantitative studies on cutaneous erythema induced by ultraviolet radiation. *Brit J Dermatol* 1984; **111:** 673–82.

15 Farr PM, Diffey BL. The erythemal response of human skin to ultraviolet radiation *Br J Dermatol* 1985; **113:** 65–76.

16 Kaidbey KH. Acquired photoprotection from UVA-induced melanogenesis. In: *The Biological Effects of UVA Radiation* (Urbach F, Gange RW, eds). New York: Praeger Publishers, 1986; 175–81.

17 Kaidbey KH, Kligman AM. Sunburn protection by long wave ultraviolet radiation-induced pigmentation. *Arch Dermatol* 1978; **114:** 46–8.

18 Rosario R, Mark GJ, Parrish JA, Mihm MC. Histological changes produced in skin by equally erythemogenic doses of UVA, UVB, UVC and UVA with psoralens. *Brit J Dermatol* 1979; **101:** 299–308.

19 Parrish JA, Jaenicke KF, Morison WL *et al.* Solar urticaria. treatment with PUVA and mediator inhibitors. *Br J Dermatol* 1982; **106:** 575–80.

20 Hindson C, Spiro J, Downey A PUVA therapy of chronic actinic dermatitis. *Br J Dermatol* 1985; **113:** 157–60.

21 Friedmann PS. Disappearance of epidermal Langerhans cells during PUVA therapy. *Br J Dermatol* 1981; **105:** 219–21.

22 Morison WL, Parrish JA, Bloch KJ, Krugler JI Transient impairment of peripheral blood lymphocyte function during PUVA therapy. *Br J Dermatol* 1979; **101:** 391–7.

23 Veronese FM, Schiavon O, Bevilacqua R *et al.* Photoinactivation of enzymes by linear and angular furocoumarins. *Photochem Photobiol* 1982; **36:** 25–30.

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Skin lesions associated with circulating lupus anticoagulant

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SUMMARY

This retrospective study of 295 patients extends the earlier findings of an association between lupus anticoagulation and thrombosis by demonstrating the occurrence of cutaneous lesions related to the coagulation defect in 70 patients. The most frequent cutaneous associations were thrombophlebitis, skin ulcer, gangrene, haemorrhage, and cutaneous necrosis. Of the patients with skin lesions, 41% had the skin lesion as the first sign of the disease. It is important to recognize this association because nearly 40% of the patients with skin lesions have multisystem thrombotic phenomena in the course of the disease.

Circulating endogenous anticoagulants in patients with systemic lupus erythematosus (SLE) were first recognized by Conley and Hartmann in 1952.[1,2] The term 'lupus anticoagulant (LA)' was introduced in 1972 by Feinstein and Rapaport.[3] LA is considered to be a group of immunoglobulins, usually IgG or IgM, that interfere to a variable extent in various phospholipid-dependent coagulation tests without inhibiting the activity of specific coagulation factors.[4–6] LA appears to react specifically with the phospholipid portion of the prothrombin activator complex, including factor Xa, factor V, phospholipid, and ionized calcium.

The presence of circulating LA is detected in the laboratory by different techniques. The hallmark of lupus anticoagulation is prolongation of phospholipid-dependent coagulation and lack of correction by 1:1 mixture with normal plasma. This may affect the prothrombin time, partial thromboplastin time, plasma clot time, and thrombin time.

Initially, LA was observed in patients with SLE[7–9] at an overall frequency between 4% and 10%.[7,10,11] Later, the inhibitor was found in patients without SLE;[12] some of these patients had other autoimmune disorders[13–15] or carcinoma[15,16] or were taking drugs such as hydralazine, chlorpromazine, phenothiazine, and procainamide.[17–20] There have been a few reports of familial occurrence of LA[21,22] and occurrence in a normal population.[23] Recently, it

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has been recognized that a high percentage of patients with acquired immunodeficiency syndrome (AIDS) have circulating LA.[24,25]

The presence of LA is associated with certain clinical and biological manifestations including a false-positive serologic test for syphilis,[26] recurrent abortion,[27-32] and thromboembolic phenomena.[33-37] An association between LA and thrombosis was first recognized by Bowie *et al.* in 1963[33] and subsequently reported in other patients.[34] Thromboembolic phenomena have been reported in 8–12% of patients with SLE but without LA,[38,39] but in patients with LA with or without SLE the frequency of this complication is higher, between 25% and 58%.[19,40,41]

Reported skin lesions include leg ulcers,[42-44] cutaneous gangrene,[45-48] thrombosis of dermal vessels,[49] widespread cutaneous necrosis,[50,51] thrombophlebitis,[19,52] necrotizing purpura,[43] nail fold vasculitis,[53] and livedo reticularis.[53,54]

The purpose of the study reported here was to determine whether or not the presence of circulating LA was associated with cutaneous lesions and the relationship of LA to other systemic manifestations.

METHODS

From about 2,000 patients screened per year between 1960 and 1982, 295 patients were found to have the lupus-like circulating anticoagulant (LA) and their medical records were reviewed. The clinical, laboratory, and histopathologic data from the patients with skin manifestations were studied in detail.

The diagnosis of LA was established according to previously described criteria[55] including: (i) prolongation of the plasma–clot time (recalcification clotting time of platelet-rich plasma) more than 5 sec above the normal range and lack of correction to normal in a 1:1 mixture with normal platelet-rich plasma and (ii) similar prolongation of the partial thromboplastin time (activated or nonactivated) and lack of correction in a 1:1 mixture with normal platelet-rich plasma. An additional criterion was the lack of inhibition of a single coagulation factor. For our study, we grouped the patients as those having SLE or lupus-like disease and those without SLE.

Histologic material from the skin lesion was available from 25 patients. The specimens were fixed in formalin, processed in paraffin, and stained with haematoxylin-eosin and periodic acid-Schiff stain.

Frozen specimens for direct immunofluorescence studies were available from 20 patients. Immunofluorescence staining of skin biopsy specimens was performed as described[56] with fluorescein conjugates of monospecific goat immunoglobulins to human IgG, IgM, IgA, C3, and fibrinogen.

RESULTS

Of the 295 patients with circulating LA studied, 70 presented with skin manifestations: 39 (56%) were females and 31 (44%) were males; mean age was 39 years (range, 16–72 years).

The most frequent cutaneous manifestation was a spontaneous thrombophlebitis syndrome, present in 34% of the patients (Table 1). This consisted of oedema and cutaneous erythema accompanied by inflammatory changes. Most of the lesions were located on the lower leg, ankle, and calf.

Twenty-one (30%) patients had painful, sharply marginated skin ulcers, usually in the pretibial area and ankle (Fig. 1). The ulcers often were multiple and focal and had a necrotic

TABLE I. Skin lesions in patients with LA

Lesion	Total patients		No. with SLE	No. without SLE
	No.	%		
Thrombophlebitis	24	34	15	9
Skin ulcer	21	30	7	14
Gangrene	13	19	5	8
Haemorrhage	10	14	4	6
Cutaneous necrosis	2	3	2	0
Total	70		33	37

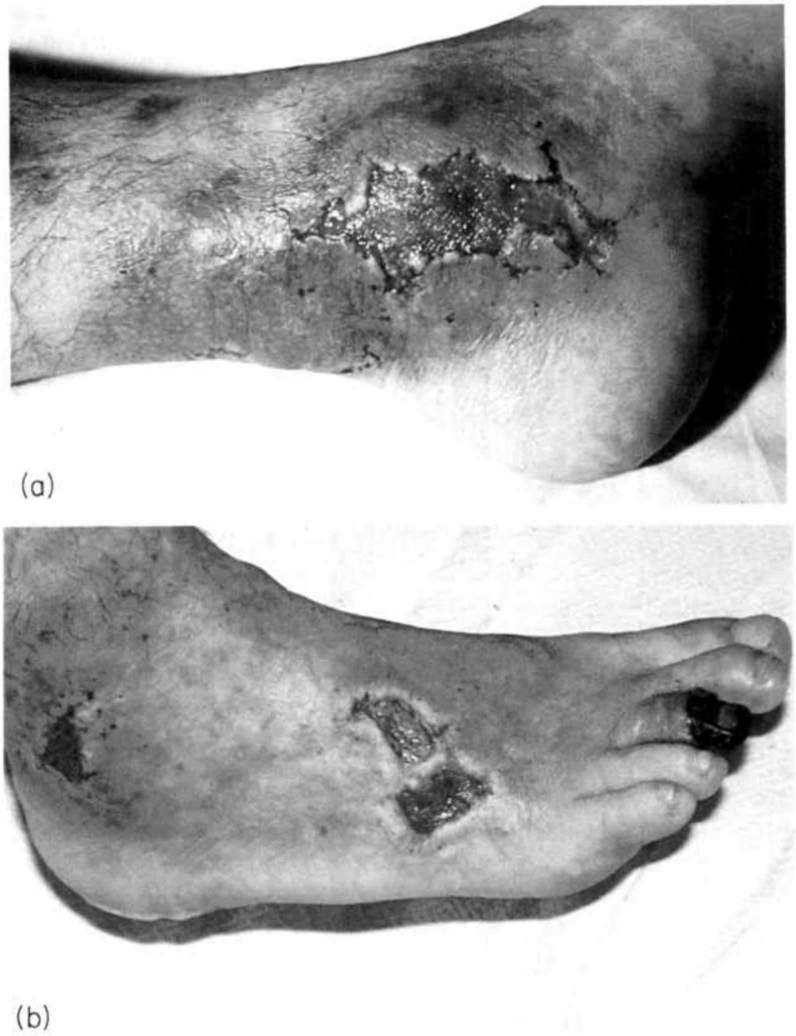

FIGURE I. Skin ulcers in patients with circulating LA: (a) stellate lesion; (b) associated gangrene of third toe.

centre or base. The periphery of the ulcer was non-inflammatory. The ulcers healed with white atrophic scars.

Twelve patients (14%) had purpura or ecchymoses and two patients had subcutaneous haematoma. The major cutaneous sign was the presence of gangrene of the digits (Fig. 1(b)). Digital gangrene progressed to require surgical amputation in two patients. Two patients presented with widespread cutaneous necrosis. There were two or more cutaneous manifestations in 25% of the patients, including livedo reticularis in five cases and Raynaud's phenomenon in three cases.

Histopathologic study of skin biopsy specimens from 25 patients detected thrombosis of small dermal vessels in 18 of of the cases (Figs 2 and 3). Haemorrhage and dermal haemosiderin deposits were present in eight and 10 of the cases, respectively. Endarteritis obliterans was observed in 10 cases and papillary capillary proliferation, in eight (Fig. 4). In the two patients with widespread cutaneous necrosis, severe infarctive haemorrhagic necrosis of the dermis and epidermis was observed.

Direct immunofluorescence studies of lesional skin, performed in 20 cases, gave positive, non-specific results in 15 cases. Immunoreactant deposits in dermal vessels were found in eight cases, including fibrinogen in five cases, IgM in four cases, C3 in three cases, and IgG in one case. In the patients with widespread cutaneous necrosis, fibrinogen deposits were observed in the necrotic epidermis, even in isolated keratinocytes. Basement membrane immunoreactants (lupus band test) were detected in lesional skin from five patients, all known to have clinical SLE with LA.

Immunologic tests

A false-positive syphilis serologic test was found in 43% of the patients (Table 2); it was more frequent in patients with SLE (45%) than in those without (34%). This test was positive more often among the patients with skin ulcer and thrombophlebitis than among those with haemorrhage and infarction (47–55% vs. 14–21%). All but three of the patients with antinuclear or anti-native-DNA antibodies at abnormal titers had SLE; three patients without SLE had antinuclear antibodies at low titers. Cryoglobulins were absent in all 38 patients tested.

Peripheral vascular studies

Radiologic vascular studies confirmed the thrombotic phenomena in 25 patients. Venography, performed in 12 patients, showed deep vein thrombosis or incompetence of deep veins secondary to thrombosis in all 12. In 10 patients, arteriography was performed, and arterial thrombosis was found in all of them (Fig. 5). In three patients, both arterial and venous thrombotic occlusion was confirmed by angiography.

Associated thrombotic phenomena

Of the patients with skin lesions, 26 (37%) showed one or more thrombotic phenomena in organs apart from the skin (Table 3). Ten patients had more than one associated thrombotic event. Vascular thrombosis in the eye was associated with cerebral infarction in three cases and with ischemic necrosis of bone and myocardial infarction in one case each. One patient with cutaneous thrombosis also had cerebrovascular infarction, myocardial infarction, and thrombotic retinal vessels. Cerebral infarction was present in two patients with renal vascular thrombosis and one with myocardial infarction. One patient with splenic infarction died 5 days after splenectomy. Autopsy showed both hepatic artery thrombosis and an old thrombotic

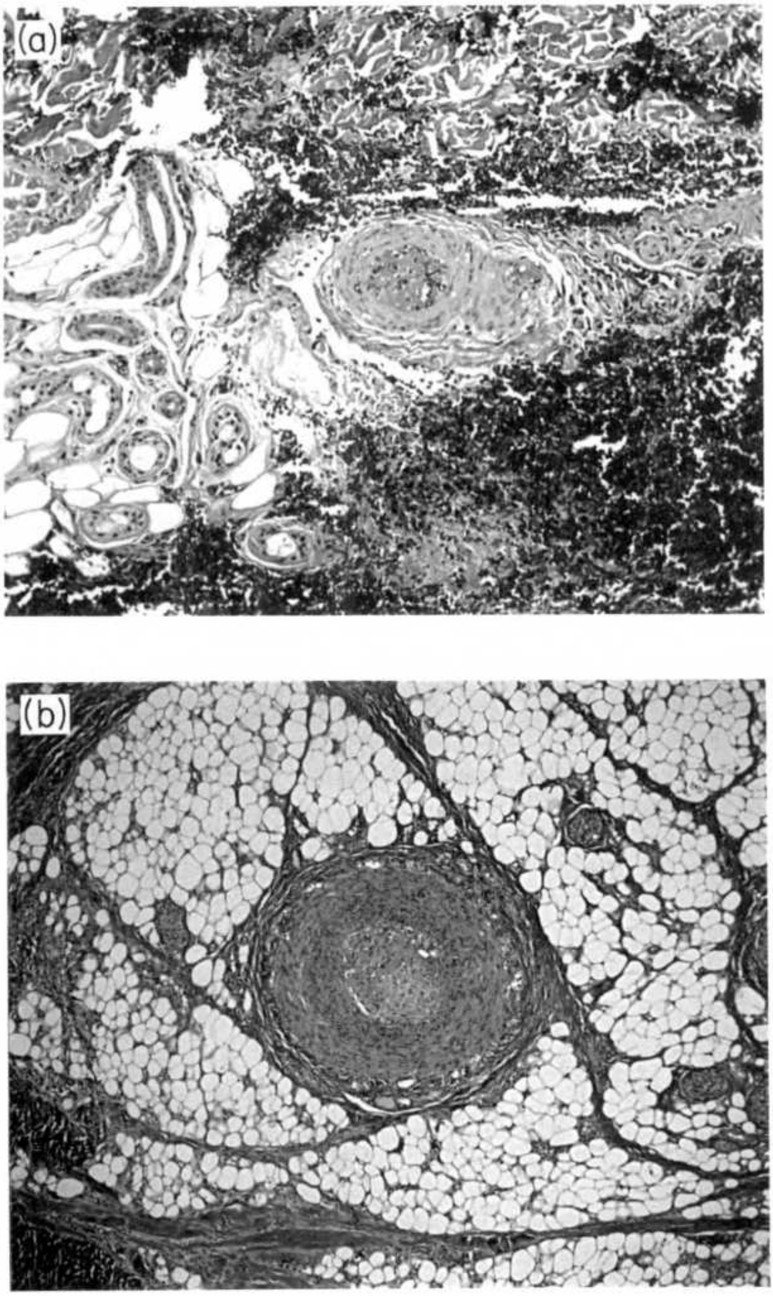

FIGURE 2. Thrombosis (Haematoxylin and eosin; × 54.4): (a) in deep dermal vessels with haemorrhage; (b) in an artery in the finger of a patient with gangrene associated with LA.

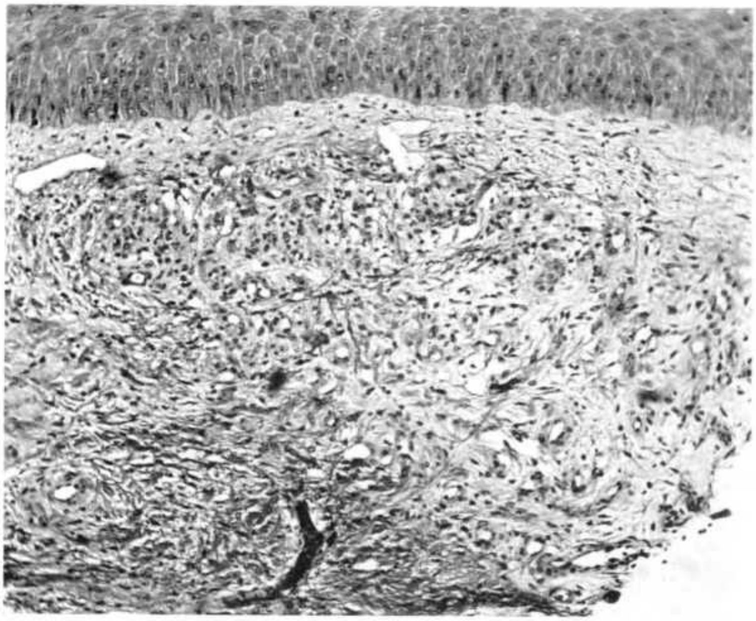

FIGURE 3. Arcades of capillary proliferation in subpapillary dermis. (Haematoxylin and eosin; × 136.)

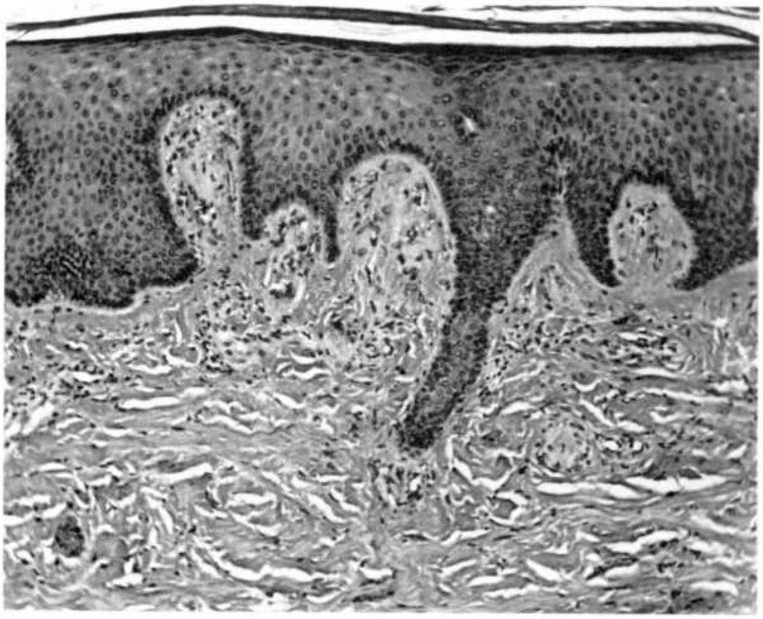

FIGURE 4. Papillary dermal vessels occluded with hyaline thrombi associated with haemorrhage. (Haematoxylin and eosin; × 136).

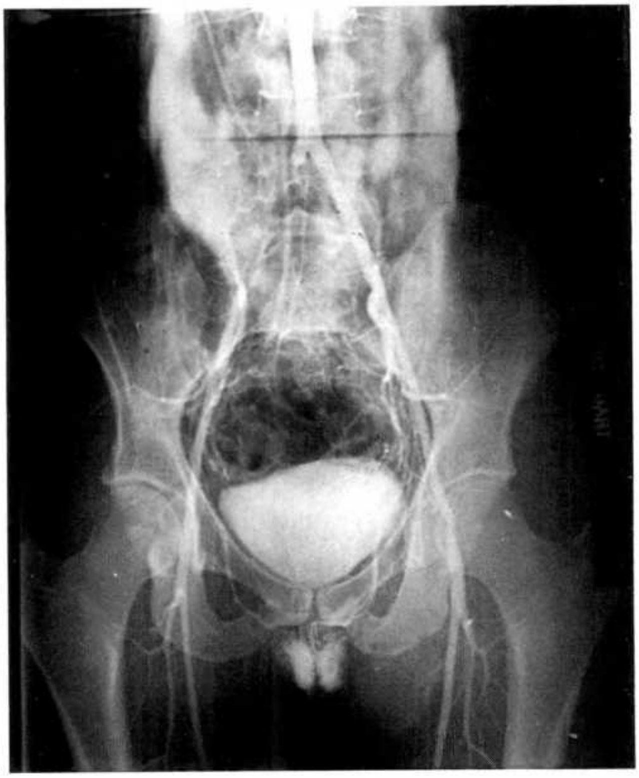

FIGURE 5. Aortogram showing complete occlusion of left iliac artery.

TABLE 2. Results of serologic studies in patients with circulating LA and skin lesions

	% with abnormal test* result		
Patients	VDRL	ANA	ADNA
Entire group	43	57	60
SLE group	45	90	92
Without SLE	34	4	0

* Tests: VDRL, Venereal Disease Research Laboratory serologic test for syphilis; ANA, antinuclear antibody test; ADNA, anti-native-DNA antibody test.

pulmonary vessel. Nine cases of pulmonary embolism and one case of pulmonary hypertension were found.

DISCUSSION

A significant number of patients with circulating LA have cutaneous lesions: superficial thrombophlebitis, skin ulcers, infarction, and haemorrhage. Nearly half of the patients have the

TABLE 3. Systemic thrombotic phenomena in LA patients with skin lesions

Thrombotic phenomenon	No.	No. with SLE
Retinal vessel occlusion	12	6
Cerebrovascular stroke	8	5
Renal vessel thrombosis	7	6
Ischaemic bone necrosis	3	3
Myocardial infarction	3	2
Splenic infarction	2	1
Hepatic artery thrombosis	1	0

cutaneous lesions before detection of either LA or SLE, providing an opportunity for early clinical diagnosis.

The mean age in our series, 39 years, is similar to the mean in published cases of LA and thrombosis.[40,57] Cutaneous lesions were slightly more common in women (56%) than in men (44%); however, LA is much more common in women, with a female-to-male ratio of 4:1 to 5:1.[40,58] Nevertheless, some authors believe that the incidence of thrombotic phenomena in patients with LA is higher in males.[59] The high incidence of cutaneous lesions with thrombosis associated with the presence of circulating LA confirms the increased risk to males with LA of having thrombotic phenomena.

Superficial thrombophlebitis was present in 34% of our patients with skin lesions. The incidence of thrombophlebitis in patients with SLE is between 0·4% and 12%.[60] We found a much higher incidence in patients with circulating LA and skin lesions, with a slightly higher incidence in patients with SLE than in those without (45% vs. 24%).

Skin ulcers have been reported in patients with SLE[61] as well as in patients with circulating LA.[42] The ulcers usually developed around and above the ankles and, in some cases, on the calf and elbow.

Thirteen patients had cutaneous gangrene and necrosis of the digits. Jungers *et al.*[62] reported arterial occlusion in seven patients with SLE, and in four of them it developed early in the course of the disease. Development of arterial thrombosis is a well-recognized phenomenon in SLE and may appear early in the course of the disease.[63] It can be the result of vasculitis, cryoglobulinaemia, or LA. All of our patients tested were negative for cryoglobulinaemia.

Despite the significant alterations in the clotting mechanism, bleeding in patients with circulating LA is rare and usually is associated with thrombocytopenia or hypoprothrombinaemia.[64] Eight percent of the patients presented with haemorrhage in the form of purpura, ecchymosis, and subcutaneous haematoma but in only three was it possible to demonstrate another coagulation defect—thrombocytopenia in all three cases.

Histopathologic study of skin revealed, in most cases, changes probably related to the coagulation defect. The presence of thrombosed vessels in 18 of 25 biopsies may be considered characteristic of the skin lesions in patients with LA, but it is not specific because it is found in other disorders such as livedoid vasculitis, thrombocythaemia, embolism, and arteriosclerosis. Nevertheless, the presence of hyaline thrombosed vessels must alert the pathologist to the possibility of circulating LA.

Of particular interest in the present series is the high incidence of systemic thrombotic phenomena. Twenty-six patients with skin lesions and LA showed thrombotic phenomena in other organs. This is similar to the frequency of systemic thrombotic phenomena reported[13] in patients with circulating LA and is significantly higher than that found in patients with SLE but without LA. The most frequent systemic involvements were retinal vessel occlusion, cerebrovascular stroke, and renal vessel occlusion. There are recent reports[19,40,65] describing similar multisystem thrombosis in patients with circulating LA.

Increased anticardiolipin levels have been found in more than 90% of SLE patients with LA. The highest anticardiolipin levels were associated with a high incidence of thrombosis.[66] This finding suggests that the presence of high levels of anticardiolipin antibodies may be a useful marker for risk of thrombosis in patients with LA[40,67,68] and may define a unique subgroup of patients with thrombotic phenomena.[13,69] An increase in anticardiolipin antibodies also has been found in patients with extensive livedo reticularis related to SLE.[70] Further efforts to detect antibodies to identify patients at risk for thrombosis may need to incorporate additional techniques; perhaps specific assays for phospholipid or cardiolipin antibodies will help. Because a significant number of patients with LA may have skin lesions as a manifestation of their immunologic disease, it is important to test for LA in patients with a thromboembolic cutaneous manifestation for which no other pathogenic mechanism is apparent.

REFERENCES

1 Conley CL Disorders of the blood in disseminated lupus erythematosus (editorial). *Am J Med* 1952; **13:** 1–2.

2 Conley CL, Hartmann RC. A haemorrhagic disorder caused by circulating anticoagulant in patients with disseminated lupus erythematosus (abstract). *J Clin Invest* 1952; **31:** 621–2.

3 Feinstein DI, Rapaport SI Acquired inhibitors of blood coagulation. *Prog Hemost Thromb* 1972; **1:** 75–95.

4 Brandt JT, Triplett DA, Musgrave K, Orr C. The sensitivity of different coagulation reagents to the presence of lupus anticoagulants. *Arch Pathol Lab Med* 1987; **111:** 120–4.

5 Exner T. Similar mechanism of various lupus anticoagulants. *Thromb Haemost* 1985; **53:** 15–8.

6 Triplett DA, Brandt JT, Maas RL. The laboratory heterogeneity of lupus anticoagulants. *Arch Pathol Lab Med* 1985, **109:** 946–51.

7 Lee SL, Miotti AB. Disorders of hemostatic function in patients with systemic lupus erythematosus. *Semin Arthritis Rheum* 1975; **4:** 241–52.

8 Lee SL, Sanders M. A disorder of blood coagulation in systemic lupus erythematosus. *J. Clin Invest* 1955; **34:** 1814–22.

9 Veltkamp JJ, Kerkhoven P, Loeliger EA. Circulating anticoagulant in disseminated lupus erythematosus. Proposed mode of action. *Haemostasis* 1973; **2:** 253–9.

10 Duran-Suarez JR. Anticoagulantes circulantes en el lupus eritematoso sistemico. *Med Clin (Barc)* 1983; **81:** 203–6.

11 Regan MG, Lackner H, Karpatkin S. Platelet function and coagulation profile in lupus erythematosus. Studies in 50 patients. *Ann Intern Med* 1974; **81:** 462–8.

12 Frick PG. Acquired circulating anticoagulants in systemic 'collagen disease': auto-immune thromboplastin deficiency. *Blood* 155; **10:** 691–706.

13 Boey ML, Colaco CB, Gharavi AE *et al.* Thrombosis in systemic lupus erythematosus: striking association with the presence of circulating lupus anticoagulant. *Br Med J [Clin Res]* 1983; **287:** 1021–3.

14 Boxer M, Ellman L, Carvalho A. The lupus anticoagulant. *Arthritis Rheum* 1976; **19:** 1244–8.

15 Exner T, Rickard KA, Kronenberg H. A sensitive test demonstrating lupus anticoagulant and its behavioural patterns. *Br J Haematol* 1978; **40:** 143–51.

16 Sidor CF, Martin SE, Abel R, Blatt PM. Lupus anticoagulants. *Del Med J* 1986; **58:** 239–45.

17 Bell WR, Boss GR, Wolfson JS. Circulating anticoagulant in the procainamide-induced lupus syndrome. *Arch Intern Med* 1977; **137:** 1471–3.

18 Canoso RT, Sise HS. Chlorpromazine-induced lupus anticoagulant and associated immunologic abnormalities. *Am J Hematol* 1982; **13:** 121–9.

19 Mueh JR, Herbst KD, Rapaport SI. Thrombosis in patients with the lupus anticoagulant. *Ann Intern Med* 1980; **92:** 156–9.

20 Zarrabi MH, Zucker S, Miller F *et al.* Immunologic and coagulation disorders in chlorpromazine-treated patients. *Ann Intern Med* 1979; **91:** 194–9.

21 Exner T, Barber S, Kronenberg H, Rickard KA. Familial association of the lupus anticoagulant. *Br J Haematol* 1980; **45:** 89–96.

22 Hermansky F, Demjal S. Anticoagulant familial in activité antithromboplastique. *Nouv Rev Fr Haematol* 1959; **14:** 12.

23 Duran-Suarez JR. Incidence of circulating anticoagulants in normal population. *Acta Haematol (Basel)* 1982, **67:** 217–9.

24 Bloom EJ, Abrams DI, Rodgers G. Lupus anticoagulant in the acquired immunodeficiency syndrome. *J. Am Med Assoc* 1986; **256:** 491–3.

25 Cohen AJ, Philips TM, Kessler CM. Circulating coagulation inhibitors in the acquired immunodeficiency syndrome. *Ann Intern Med* 1986; **104:** 175–80.

26 Harvey AM, Shulman LE. Systemic lupus erythematosus and the chronic false-positive test for syphilis. In: *Lupus Erythematosus: A Review of the Current Status of Discoid and Systemic Lupus Erythematosus and Their Variants* (Dubois EL, ed), 2nd edn. Los Angeles: University of Southern California Press, 1974; 196.

27 Carreras LO, Defreyn G, Machin SJ *et al.* Arterial thrombosis, intrauterine death and 'lupus' anticoagulant: detection of immunoglobulin interfering with prostacyclin formation. *Lancet* 1981; **i:** 244–6.

28 de Castellarnau C, Vila L, Sancho MJ *et al.* Lupus anticoagulant, recurrent abortion, and prostacyclin production by cultured smooth muscle cells (letter to the editor). *Lancet* 1983; **ii:** 1137–8.

29 Duran-Suarez JR, Pigrau-Serrallach C, Bosch-Gil J *et al.* Possible relationship between circulating anticoagulants and recurrent abortion. *Haematologica (Pavia)* 1982; **67:** 320–1.

30 Feinstein DI. Lupus anticoagulant, thrombosis, and fetal loss (editorial). *N Engl J Med* 1985; **313:** 1348–50.

31 Firkin BG, Howard MA, Radford N. Possible relationship between lupus inhibitor and recurrent abortion in young women (letter to the editor). *Lancet* 1980; **ii:** 366.

32 Petri M, Rheinschmidt M, Whiting-O'Keefe Q *et al.* The frequency of lupus anticoagulant in systemic lupus erythematosus: a study of sixty consecutive patients by activated partial thromboplastin time, Russell viper venom time, and anticardiolipin antibody level *Ann Intern Med* 1987, **106:** 524–31.

33 Bowie EJW, Thompson JH Jr, Pascuzzi CA, Owen CA Jr. Thrombosis in systemic lupus erythematosus despite circulating anticoagulants. *J Lab Clin Med* 1963; **62:** 416–30.

34 Elias M, Eldor A. Thromboembolism in patients with the 'lupus'-type circulating anticoagulant. *Arch Intern Med* 1984; **144:** 510–5.

35 Landi G, Calloni MV, Grazia Sabbadini M *et al* Recurrent ischemic attacks in two young adults with lupus anticoagulant. *Stroke* 1983; **14:** 377–9.

36 Lubbe WF, Butler WS, Palmer SJ, Liggins GC. Lupus anticoagulant in pregnancy. *Br J Obstet Gynaecol* 1984; **91:** 357–63.

37 Lupus anticoagulant (editorial). *Lancet* 1984; **i:** 1157–8.

38 Angles-Cano E, Sultan Y, Clauvel JP. Predisposing factors to thrombosis in systemic lupus erythematosus: possible relation to endothelial cell damage. *J Lab Clin Med* 1979; **94:** 312–23.

39 Gladman DD, Urowitz MB. Venous syndromes and pulmonary embolism in systemic lupus erythematosus. *Ann Rheum Dis* 1980; **39:** 340–3.

40 Lechner K, Pabinger-Fasching I. Lupus anticoagulants and thrombosis. A study of 25 cases and review of the literature. *Haemostasis* 1985; **15:** 254–62.

41 Schleider MA, Nachman RL, Jaffe EA, Coleman M. A clinical study of the lupus anticoagulant. *Blood* 1976, **48:** 499–509.

42 Bazex A, Bazex J, Boneu B *et al.* Ulcere de jambe, anticoagulant circulant. Lupus érythémateaux aigu disséminé. *Bull Soc Fr Dermatol Syph* 1976; **83:** 350–2.

43 Johansson EA, Niemi KM, Mustakallio KK. A peripheral vascular syndrome overlapping with systemic lupus erythematosus. Recurrent venous thrombosis and hemorrhagic capillary proliferation with circulating anticoagulants and false-positive seroreactions for syphilis. *Dermatologica* 1977; **155:** 257–67.

44 Cuny JF, Schmutz JL, Jeandel C *et al.* Ulcère de jambe et anti-coagulants circulants. *Ann Dermatol Venereol* 1986; **113:** 825–6.

45 Godeau P, Bletry O, Piette JC, Wechsler B. Anticoagulants circulants. Les conditions cliniques du diagnostic. *Rev Med Interne* 1985; **6:** 523–41.

46 Jindal BK, Martin MF, Gayner A. Gangrene developing after minor surgery in a patient with undiagnosed systemic lupus erythematosus and lupus anticoagulant. *Ann Rheum Dis* 1983; **42:** 347–9.

47 Asherson RA, Derksen RHWM, Harris EN *et al.* Large vessel occlusion and gangrene in systemic lupus erythematosus and 'lupus-like' disease. A report of six cases. *J Rheumatol* 1986; **13:** 740–7.

48 Bird AG, Lendrum R, Asherson RA, Hughes GRV. Disseminated intravascular coagulation, antiphospholipid antibodies, and ischaemic necrosis of extremities. *Ann Rheum Dis* 1987; **46:** 251–5.

49 Grob JJ, Bonerandi JJ. Cutaneous manifestations associated with the presence of the lupus anticoagulant. A report of two cases and a review of the literature. *J Am Acad Dermatol* 1986; **15:** 211–9.

50 Dodd HJ, Sarkany I, O'Shaughnessy D. Widespread cutaneous necrosis associated with the lupus anticoagulant. *Clin Exp Dermatol* 1985; **10:** 581–6.

51 Francès C, Boisnic S, LeFebvre Ch *et al.* Manifestations cutanées rares au cours du lupus: nécrose cutanée étendue superficielle. *Ann Dermatol Venereol* 1986; **113:** 976–7.

52 Olive D, Andre E, Brocard O *et al.* Lupus erythemateux dissemine revele par des thrombophlebites des membres inferieurs. *Arch Fr Pediatr* 1979; **36:** 807–11.

53 Williams H, Laurent R, Gibson T. The lupus coagulation inhibitor and venous thrombosis: a report of four cases. *Clin Lab Haematol* 1980; **2:** 139–44.

54 White CR Jr, Hall TL. Dermatopathology of cutaneous changes associated with lupus anticoagulant (abstract). *J Cutan Pathol* 1986; **13:** 93.

55 Gastineau DA, Kazmier FJ, Nichols WL, Bowie EJ. Lupus anticoagulant: an analysis of the clinical and laboratory features of 219 cases. *Am J Hematol* 1985; **19:** 265–75.

56 Coons AH, Creech HJ, Jones RN, Berliner E. The demonstration of pneumococcal antigen in tissues by the use of fluorescent antibody. *J Immunol* 1942; **45:** 159–70.

57 Durán Suárez JR, Trigmer J. Aspectos clinico-biológicos de las antiprotrombinas: estudio de siéte casos. *Sangre* 1982; **27:** 994–1002.

58 Eldor A, Elias M. Thromboembolic events in patients with 'lupus' type anticoagulant (abstract). *Thromb Haemost* 1983; **50:** 345.

59 Waddell CC, Brown JA. The lupus anticoagulant in 14 male patients. *J Am Med Assoc* 1982; **248:** 2493–5.

60 Peck B, Hoffman GS, Franck WA. Thrombophlebitis in systemic lupus erythematosus. *J Am Med Assoc* 1978; **240:** 1728–30.

61 Alarcón-Segovia D, Osmundson PJ. Peripheral vascular syndromes associated with systemic lupus erythematosus. *Ann Intern Med* 1965; **62:** 907–19.

62 Jungers P, Dougados M, Gazengel C *et al.* Manifestations thromboemboliques au cours du lupus erythemateaux dissemine. *Ann Med Interne (Paris)* 1980; **131:** 416–21.

63 Dubois EL, Arterberry JD. Gangrene as a manifestation of systemic lupus erythematosus. *J Am Med Assoc* 1962; **181:** 366–74.

64 Mannucci PM, Canciani MT, Mari D, Meucci P. The varied sensitivity of partial thromboplastin and prothrombin time reagents in the demonstration of the lupus-like anticoagulant. *Scand J Haematol* 1979; **22:** 423–32.

65 Anderson NE, Ali MR. The lupus anticoagulant, pulmonary thromboembolism, and fatal pulmonary hypertension. *Ann Rheum Dis* 1984; **43:** 760–3.

66 Colaco CB, Male DK. Anti-phospholipid antibodies in syphilis and a thrombotic subset of SLE: distinct profiles of epitope specificity. *Clin Exp Immunol* 1985; **59:** 449–56.

67 Hughes GR. Thrombosis, abortion, cerebral disease, and the lupus anticoagulant (editorial). *Br Med J [Clin Res]* 1983; **287:** 1088–9.

68 Lockshin MD, Druzin ML, Goei S *et al.* Antibody to cardiolipin as a predictor of fetal distress or death in pregnant patients with systemic lupus erythematosus. *N Engl J Med* 1985; **313:** 152–6.

69 Hughes GR, Harris NN, Gharavi AE. The anticardiolipin syndrome. *J Rheumatol* 1986; **13:** 486–9.

70 Weinstein C, Miller MH, Axtell R *et al.* Livedo reticularis associated with increased titers of anticardiolipin antibodies in systemic lupus erythematosus. *Arch Dermatol* 1987; **123:** 596–600.

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Solitary morphoea profunda

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SUMMARY

This study describes a series of patients with an unusual form of scleroderma. All five patients presented clinically with solitary, indurated plaques involving the upper trunk. Histologically the lesions were characterized by a dense, mononuclear cell infiltrate mainly in the subcutis, which was rich in plasma cells, and was associated with marked sclerosis and hyalinization of the connective tissue at this level. Lymphoid aggregates with germinal centre formation were present in three patients and tissue eosinophilia in one. No lesion has shown spontaneous resolution but neither has systemic involvement occurred, despite a maximum follow-up of 17 years. Immunohistochemical studies revealed a mixed population of T and B lymphocytes with no evidence of immunoglobulin light-chain restriction. We believe that the clinical and histological features in these five cases are sufficiently distinct to merit description under the title of 'solitary morphoea profunda'.

Morphoea is considered to be a purely cutaneous form of scleroderma and has traditionally been classified into various subgroups: guttate, plaque, linear, segmental and generalized.[1] Histologically the condition is characterized by an inflammatory and sclerotic phase, with most cases showing a mixture of both features.[1] Apart from superficial morphoea, there is usually full thickness involvement of the dermis with some extension into the subcutaneous tissue.[2,3] Recently Person and Su described a group of 23 patients with generalized morphoea in whom a dense inflammatory cell infiltrate in the subcutis and underlying fascia was associated with extensive sclerosis and hyalinization of the connective tissue at this level.[4,5] The term 'morphoea profunda' was suggested for these cases. The five cases reported here are histologically similar but clinically distinct. The clinicopathological and immunohistochemical results are reported under the title, 'Solitary Morphoea Profunda'.

METHODS

Patients

Five patients, three males and two females, are described with a mean age of 42 years. Full clinical details are given in Table 1. In addition to routine H and E staining, sections from four

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TABLE 1. Clinical features

Case	Sex/Age at onset	Distribution	Associated features	Duration of activity (years)	Eosinophils ($\times 10^6$/l)	ESR (mm/h)	Therapy	Miscellaneous
1 MW	F 67	Intrascapular	Dysphagia	17	100	30	Topical steroids	Barium meal—sliding hiatus hernia. CXR normal, ANF 1:10 Immunoglobulins IgA5.6g/l Prot. electrophoresis—No M band Kveim and Mantoux—negative VDRL/TPHA—negative
2 GB	F 37	Midscapular	Psoriasis	6	120	20	—	Anti-Mito Ab 1:320 LFTs—normal VDRL/TPHA normal CXR—normal Immunoglobulins and protein electrophoresis—normal
3 IK	M 40	Upper back	—	7	—	—	—	Immunoglobulins and protein electrophoresis—normal Lost to follow up
4 MS	M 39	Left shoulder (post aspect)	—	12	95	—	—	VDRL and autoantibodies negative Immunoglobulins and protein electrophoresis—normal
5 LA	M 21	Right cervical region (sub-mandibular) (ulceration)	—	2	120	18	Intralesional steroids	Autoantibodies, VDRL/TPHA—negative CXR normal Immunoglobulins and protein electrophoresis—normal

TABLE 2. Monoclonal antibodies

Monoclonal antibody	Specificity
1 UCHL1 (Source: Dako)	T lymphocytes (70% helper T cells, 35% suppressor cytotoxic T cells) Macrophages + cells of myeloid series
2 4KB5 (Source: Dako)	Normal mature and malignant B lymphocytes (Germinal centres weakly, Mantle zones strongly) Monocytes Plasma cells not labelled
3 L26 (Source: Dako)	Normal mature and malignant B lymphocytes (both germinal centres and Mantle zones) Reed–Sternberg cells Plasma cells not labelled

patients were stained with PAS, congo red and orcein/saffron. Paraffin-embedded tissue from these four patients and controls were also studied using a panel of monoclonal antibodies in a standard three-step immunoperoxidase procedure. These included a T-cell marker (UCHL1), B-cell markers (4KB5 and L26), and both Kappa and Lambda antisera. Monoclonal antibodies UCHL1, 4KB5 and L26 recognize epitopes of the leucocyte common antigen which are resistant to formalin fixation. Their specificities (Table 2) are now well established.[6,7]

Case 1

This 72-year-old lady (MW) first presented in 1976 with a 5-year history of an asymptomatic lesion over the interscapular region. She was well, apart from a recent history of mild dysphagia. Examination revealed a large crescentic plaque over the back with a peripheral erythematous border associated with superficial telangiectasia. The centre of the plaque had a yellowish-brown pigmentation with a hard nodular and infiltrated surface. Clinical diagnoses suggested at this stage included cutaneous lymphoma and morphoea.

An initial biopsy revealed a dense perivascular and periappendageal inflammatory cell infiltrate consisting of both mononuclear cells and plasma cells. Prominent lymphoid aggregates were noted in the subcutis and a focus of epithelioid cells and Langhans giant cells were also present in the lower dermis. There was sclerosis and hyalinization of collagen in the reticular dermis and subcutis. No definite diagnosis was established at this stage although a differential included tertiary syphilis, morphoea and lymphoma.

A subsequent biopsy from the edge of the plaque showed a more florid inflammatory infiltrate consisting of numerous lymphoid follicles with germinal centre formation in the mid and lower dermis (Fig. 1). There was evidence of a granulomatous infiltrate with sclerosis and hyalinization of collagen, particularly in the subcutis. These histological features suggested the additional possibility of an unusual necrobiotic or granulomatous disorder such as sarcoidosis.

A subsequent biopsy 1 year after presentation revealed obvious sclerosis and hyalinization of collagen in the reticular dermis extending into the subcutis. This was again associated with a prominent inflammatory cell infiltrate consisting of lymphoid aggregates at the level of the subcutis and reticular dermis and also a marked interstitial plasma cell infiltrate. There was no tissue eosinophilia. These features were felt to be most consistent with an atypical form of morphoea. The patient's condition has subsequently remained unchanged for the past 17 years.

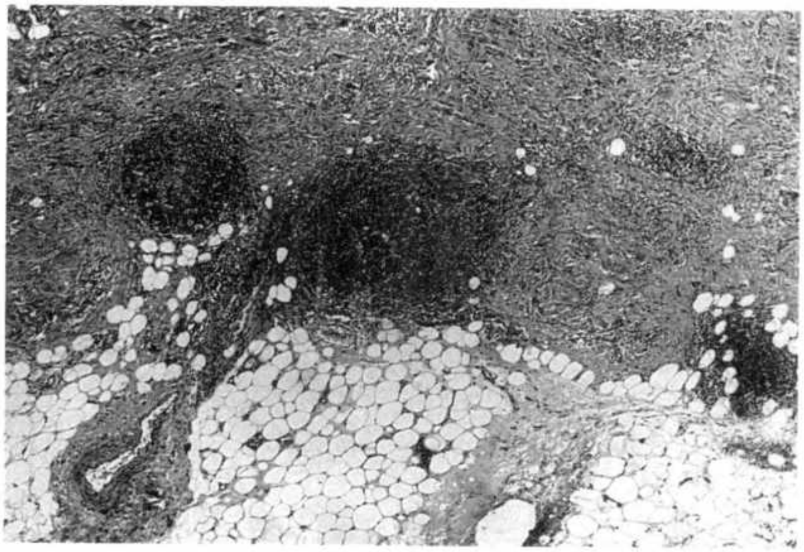

FIGURE 1. Patient 1: well defined lymphoid follicles with germinal centres at the level of the subcutis (H + E, original × 40).

Case 2

This 37-year-old female (GB) with mild flexural psoriasis presented with a 3-year history of an asymptomatic lesion involving the mid scapular region. Clinical examination revealed a raised pigmented plaque which was deeply indurated (Fig. 2). The borders were indistinct with a faint rim of telangiectasia and the surface was irregular with a peau d'orange appearance. The patient was otherwise well.

A biopsy showed a normal epidermis with marked sclerosis and hyalinization of collagen

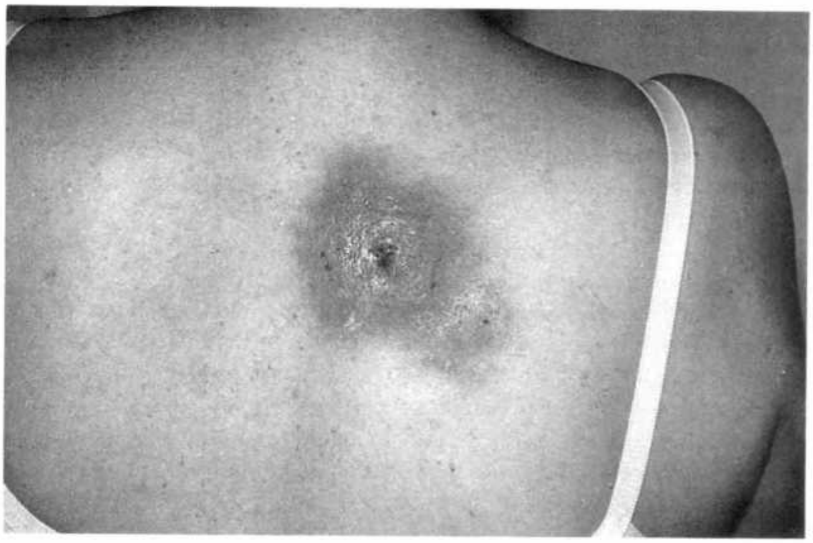

FIGURE 2. Patient 2: deeply indurated plaque upper back with peau d'orange appearance over surface.

throughout the reticular dermis and subcutis. This was associated with a mild lymphohistiocytic perivascular and periappendageal infiltrate. Numerous plasma cells were scattered diffusely through the reticular dermis. The subcutis contained a heavy lymphocytic infiltrate, but there was no tendency to form follicles or aggregates. Moderate numbers of eosinophils were present in the subcutis and reticular dermis. These features were reported as consistent with a diagnosis of morphoea, although the extent of the inflammatory infiltrate and the presence of significant tissue eosinophilia were considered to be unusual features.

Case 3

This 40-year-old male (IK) patient presented in 1981 with a hard deeply tethered plaque over the upper back. This was pigmented and the surface had a nodular infiltrated texture.

A skin biopsy revealed a mildly atrophic epidermis with pigmentary incontinence and papillary oedema. Collagen throughout the mid and lower dermis was sclerotic and hyalinized with some involvement of the subcutaneous tissue. A prominent cellular infiltrate was present at the level of the sweat coils with both plasma cell and lymphoid aggregates. Lymphoid follicles were also seen at the level of the subcutis (Fig. 3).

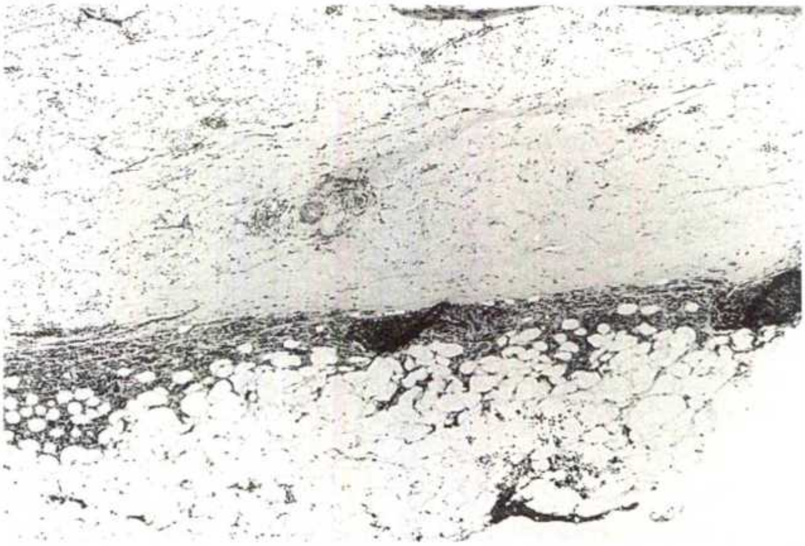

FIGURE 3. Patient 3: hyalinization of collagen in the reticular dermis with prominent cellular infiltrate at the level of the subcutis (H + E, original × 40).

Case 4

A 39-year-old male (MS) patient had had an asymptomatic erythematous lesion over the left shoulder for 5 years. This consisted of a large erythematous plaque with indistinct margins that appeared to be indurated and tethered to underlying structures. A striking peau d'orange effect was noticeable (Fig. 4) and the clinical appearances have remained unchanged for the past 7 years.

Histological sections from this patient showed a normal epidermis with marked hyalinization and sclerosis of collagen in the reticular dermis and almost complete effacement of the subcutis. This was associated with a dense, predominantly plasma, cell infiltrate situated diffusely

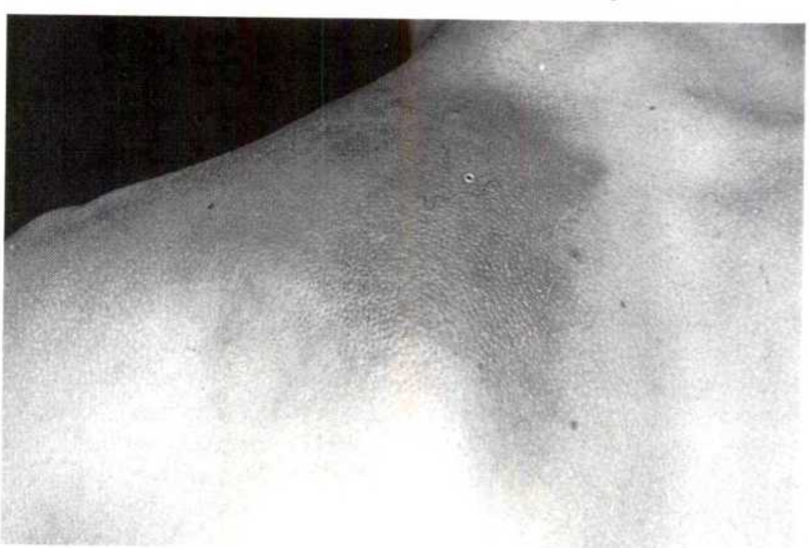

FIGURE 4. Patient 4: extensive erythematous plaque over shoulder with striking peau d'orange appearance.

through the mid and lower dermis and extending into the subcutis. There was no tissue eosinophilia and no obvious lymphoid aggregates or follicles but the extent of the inflammatory cell infiltrate was initially thought to be inconsistent with a diagnosis of simple morphoea.

Case 5

This 21-year-old male (LA) presented initially with an erythematous painless nodule under the angle of the right mandible. Over the course of 18 months this lesion gradually enlarged to form a sclerotic pale plaque with a pigmented border and superficial telangiectasia. Subsequently an

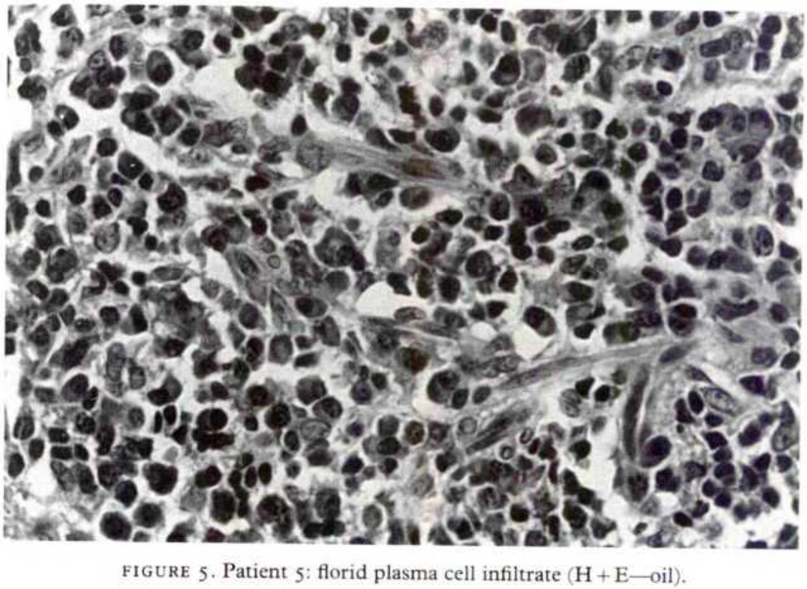

FIGURE 5. Patient 5: florid plasma cell infiltrate (H + E—oil).

area of ulceration developed at the centre of the plaque. Initial diagnoses included a soft tissue sarcoma, dermatofibrosarcoma protruberans and a reticulosis. Biopsies revealed sclerosis and hyalinization of collagen in the reticular dermis with involvement of the subcutis. This was associated with a florid interstitial mononuclear cell infiltrate throughout the dermis and subcutis which consisted predominantly of plasma cells (Fig. 5).

However, both lymphoid and plasma cell aggregates were present at the junction of the reticular dermis and subcutis. There were no lymphoid follicles but occasional eosinophils were noted.

A few large hyperchromatic plasma cells were noted in the mid dermis with occasional centrocytes, but mitoses and Russell bodies were not seen. Occasional vessels in the lower dermis showed evidence of endothelial cell hyperplasia with no obvious fibrinoid necrosis or leucocytoclasis.

A biopsy performed 1 year after presentation showed obvious hyalinization and sclerosis of collagen with a moderate perivascular and periappendigeal plasma cell infiltrate. This biopsy extended below the level of the subcutis but did not reveal involvement of the underlying fascia or muscle.

RESULTS

Special stains demonstrated that elastic tissue was fragmented and clumped in areas of sclerosis in two of the five cases. Immunohistochemical studies revealed that the inflammatory infiltrate consisted of approximately equal proportions of both T (UCHL1 + ve) and B (4KB5, L26 + ve) cells. Lymphoid follicles with germinal centres had the characteristic staining patterns of normal lymphoid tissue (mantle zones UCHL1 +, inner rim 4KB5 + and germinal centres L26 + +). Although the dermal interstitial infiltrate consisted mainly of T lymphocytes (UCHL1 +), B lymphocytes (4KB5 +, L26 +) formed the predominant cell type in the subcutis. Normal ratios of Kappa and Lambda positive cells were scattered diffusely throughout the dermis and also occasionally in aggregates at the level of the subcutis. The histological and immunohistochemical features are summarized in Table 3.

DISCUSSION

Considerable difficulty arose with the diagnosis of all these cases. The clinical features were distinct from localized plaque morphoea and alternative suggestions have included necrobiosis lipoidica, cutaneous lymphoma, dermatofibrosarcoma protruberans and syphilitic gumma. In all the patients ill-defined, indurated and deeply tethered plaques were present over the upper trunk. In three, yellowish-brown pigmentation was apparent with a nodular surface and peripheral rim of telangiectasia. Atrophy was not a feature. In two cases the plaques were initially erythematous and later developed a more characteristic pale sclerotic surface. Superficial ulceration developed in one case and in two patients a striking peau d'orange effect was noted which may have been due to upper dermal oedema. In all patients the plaques have been persistent but have shown no tendency to progress and there has been no evidence of systemic involvement despite prolonged follow-up.

The histological features have also caused difficulty in diagnosis of the condition. All the patients had extensive inflammatory cell infiltrates extending from the reticular dermis to the subcutis and plasma cells formed the predominant cell type in three of the patients. Lymphoid follicles with germinal centres were also noted in three cases and as a consequence in one patient

TABLE 3. Pathological and immunohistochemical data

	Histology				Immunohistochemistry				
Case	Collagen sclerosis Hyalinization	Plasma cell Aggregates	Lymphoid aggregates/ follicles	Tissue eosinophilia	Additional features	UCH%L1	4KB%5	L%26	Ratio of Kappa/Lambda Positive cells
1	++	++	+++	–	Epitheloid and Langhans giant cells Loss of elastin Subcutis affected	60	30	35	2:1
2	++	+/–	+	+	Subcutis affected	40	25	30	3:1
3	+	+++	++	–	Epidermal atrophy Pigmentary Incont. Papillary oedema Loss of elastin	–	–	–	–
4	++	+++	+	–	Subcutis affected	50	30	35	2:1
5	++	+++	++	+	Occasional centrocytes Endothelial cell hyperplasia No fascial or muscle involvement	50	20	40	3:2

%, % Total cellular infiltrate positive.
+ + +, Extensive.
+ +, Moderate.
+, Significant but mild.
+/–, Minimal.

(MW) a diagnosis of lymphoma was considered. However, there were no atypical mitoses and only very few hyperchromatic mononuclear cells. Sparse tissue eosinophilia was present at the level of the subcutis in two patients and a focus of epithelioid giant cells was also noted in one patient. Vascular involvement was not a characteristic feature. A diagnosis of morphoea was suggested because of the presence in all patients of extensive sclerosis and hyalinization of collagen in the reticular dermis. This sclerosis extended into the subcutis with almost complete obliteration of the panniculus in two patients. However, deep biopsies in one patient did not reveal involvement of underlying fascia and muscle.

In 1972, Fleischmajer[8] noted that full thickness biopsies from patients with generalized morphoea can reveal inflammatory changes in the adipose tissue around eccrine sweat glands and in the subcutis.[9] This infiltrate consisted of lymphocytes, plasma cells and histiocytic cells often in close aggregates. Lymphoid follicles with germinal centres were also noted in two of the five cases studied. This cellular infiltrate gradually subsided to be replaced by marked sclerosis and hyalinization of collagen. Furthermore, there was no correlation between the severity of the inflammatory infiltrate and the development of systemic involvement.[10]

More recently, Person and Su have published successive reports outlining the clinicopathological features of 23 patients; all had generalized morphoea, with the exception of two with diffuse sclerosis limited to the limbs, and one patient with a solitary plaque over the back.[4,5] In six patients the condition was progressive and associated with either pulmonary fibrosis or abnormal oesophageal motility. Hypergammaglobulinemia and peripheral eosinophilia were also present in six cases. The histological findings included sclerosis and hyalinization of collagen extending from the lower dermis into the underlying subcutis and accompanied by a marked perivascular and interstitial cellular infiltrate within the subcutaneous tissue and underlying fascia.[5] The infiltrate consisted of both lymphocytes and plasma cells and sparse tissue oesinophilia was present in only five cases. Lymphoid aggregates without germinal centre formation were described in three and focal accumulations of plasma cells were the predominant finding in five patients. We feel that the histological features of our cases conform most closely to those described by Person and Su.[5] However, apart from one of their cases,[4] the clinical features are quite distinct and none of our patients has subsequently developed evidence of systemic involvement.

Immunohistochemical studies indicated that the inflammatory infiltrates consisted of both B and T lymphocytes with no evidence of immunoglobulin light chain restriction. The distribution of B and T lymphocytes within lymphoid follicles was similar to that found in normal lymphoid tissue. However, plasma cells and B lymphocytes were the predominant cellular component at the level of the subcutis while T lymphocytes were present throughout the dermal infiltrates. The presence of intense well defined follicles with germinal centres suggests that both cellular and humoral immunity are involved in the pathogenesis of this form of atypical morphoea. Interestingly there is evidence that lymphokines can produce increased collagen production *in vitro*[11,12] but the presence of a specific antigen in scleroderma has not yet been confirmed.

Person and Su suggested that, because of histological similarities, generalized morphoea and eosinophilic fasciitis should be regarded as part of a continuous disease spectrum. Although the condition described by Shulman as eosinophilic fasciitis[13] would appear to be clinically distinct, there are reports of its association with morphoea.[14,15] However, the clinical features of our cases and the lack of histological evidence of a fasciitis does not support this contention.

In conclusion, this study emphasizes firstly that a marked inflammatory cell response with involvement of the subcutaneous tissue can occur in morphoea and, secondly, that these features are not limited to cases of generalized morphoea as previously suggested. We feel that the histological changes conform most closely to those described by Person and Su, and we therefore propose that 'solitary morphoea profunda' is the most appropriate term for this unusual clinicopathological entity.

ACKNOWLEDGMENTS

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REFERENCES

1 Lever WF, Schaumberg-Lever G. In: *Histopathology of the Skin.* Philadelphia: Lippincott, 1983; 461–7.
2 O'Leary PA, Montgomery H, Ragsdale W. Dermatohistopathology of various types of scleroderma. *Arch Dermatol* 1956; **1:** 78–87.
3 Young EM, Barr RJ. Sclerosing dermatosis. *J Clin Pathol* 1985; **12:** 426–41.
4 Person JR, Su D. Subcutaneous morphoea. *Br J Dermatol* 1979; **100:** 371–80.
5 Su D, Person JR. Morphoea profunda. *Am J Dermatopathol* 1981; **3:** 251–60.
6 Davey F, Gatter K, Ralfkiaer E *et al.* Immunophenotyping of non-Hodgkins lymphomas using a panel of antibodies on paraffin-embedded tissues. *Am J Pathol* 1987; **129:** 1, 54–63.
7 Norton AS, Issacson PG. Monoclonal antibody L26. *J Clin Pathol* 1987; **40:** 1405–12.
8 Fleischmajer R, Nedwich A. Generalized morphoea. *Arch Dermatol* 1972; **106:** 509–14.
9 Fleischmajer R, Perlish J, West W. Ultrastructure of cutaneous cellular infiltrates in scleroderma. *Arch Dermatol* 1977; **113:** 1661–6.
10 Fleischmajer R, Perlish J, Reeves J. Cellular infiltrates in scleroderma skin. *Arthritis Rheum* 1977; **20:** 975–84.
11 Johnson R, Ziff M. Lymphokine stimulation of collagen accumulation. *J Clin Invest* 1976; **58:** 240–252.
12 Spielvogel R. Mononuclear cell stimulation of fibroblast collagen synthesis. *Clin Exp Dermatol* 1978; **3:** 25–28
13 Shulman LE. Diffuse fasciitis with hypergammaglobulinaemia and eosinophilia: A new syndrome. *J Rheumatol* 1974; **1:** 46.
14 Caperton E, Hathaway D, Dehner L. Morphoea, fasciitis and scleroderma with eosinophilia: A broad spectrum of disease *Arthritis Rheum* 1976; **19:** 792–3.
15 Fleischmajer R, Jacotot A, Shore S, Binnick S. Scleroderma, eosinophilia and diffuse fasciitis. *Arch Dermatol* 1978; **114:** 1320–5.

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Sneddon's syndrome (livedo reticularis and cerebral thrombosis) with livedo vasculitis and anticardiolipin antibodies

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SUMMARY

A patient with widespread livedo reticularis and transient cerebral ischaemia (Sneddon's syndrome) is described. She also had painful scarring ulcers of the lower legs resembling livedo vasculitis and a circulating anticardiolipin antibody. We suggest that Sneddon's syndrome and livedo vasculitis may be pathogenetically related.

A patient with livedo reticularis and cerebrovascular disease was described by Champion and Rook[1] and a further six cases were subsequently documented by Sneddon.[2] The association of generalized broken livedo with transient cerebrovascular ischaemia has since become known as Sneddon's syndrome. The cause is unknown, but occlusion of small and medium-sized arteries seems to be an important feature in the pathogenesis.[3]

Livedo vasculitis is also a vaso-occlusive disorder. It is characterized clinically by a patchy livedo-like purpura with painful ulcers of the lower leg which leave white scars resembling 'atrophie blanche'.[4] The cause of livedo vasculitis is also unknown but some similar cases of painful leg ulceration have been linked with the presence of the circulating lupus anticoagulant (now known as anticardiolipin antibody).[5]

We now report a case of Sneddon's syndrome with a circulating anticardiolipin antibody and ulceration of the lower legs resembling livedo vasculitis. We suggest that these two conditions might be pathogenetically related, and the anticardiolipin antibody should be looked for in all cases of Sneddon's syndrome or livedo vasculitis.

CASE REPORT

A 47-year-old woman presented with a 10-year history of livedo reticularis of her upper arms, thighs and buttocks (Fig. 1), and painful ulceration of her lower legs with purpura and scarring

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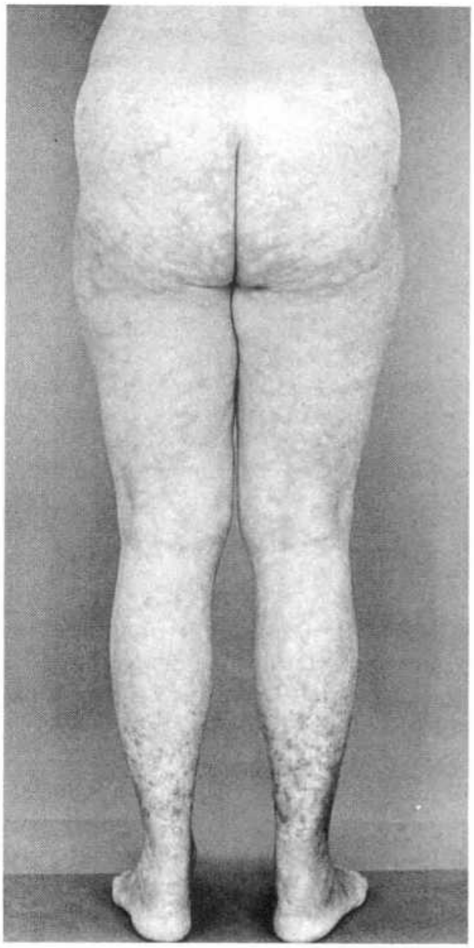

FIGURE 1. Livedo reticularis of the thighs and buttocks.

(Fig. 2). Apart from cold intolerance her general health had been good until 1983 when she developed incipient gangrene of her right second toe which resolved after two pulses of methylprednisolone. In 1987 she had an episode of transient loss of vision in the right eye followed by two episodes in the left eye, the second lasting 4 h. Examination by an ophthalmologist did not reveal any retinal abnormality but she has continued to complain of 'blank spots and wavy patterns' on that side. Prednisolone (20 mg daily) was started because the ulceration on her legs had become active again but there was no clinical improvement. However, 3 months after starting dipyridamole (50 mg t.d.s) and aspirin (75 mg o.d.) the vasculitis resolved, and did not recur when steroids were withdrawn 4 months later.

There was a past history of viral meningitis and cholecystectomy for chronic cholecystitis but no hypertension, deep vein thromboses, or thrombocytopenia. She had had three normal pregnancies with no spontaneous abortions.

Skin biopsy from the livedo reticularis on her thigh showed intimal hyperplasia of a deep

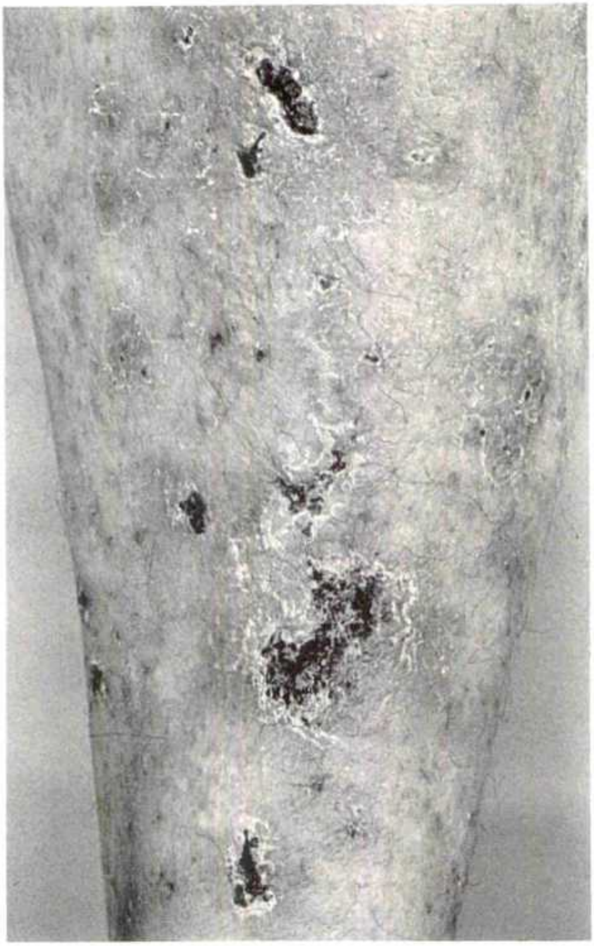

FIGURE 2. Ulceration, purpura and scarring of the lower leg.

dermal arteriole but was otherwise normal. A biopsy from the edge of a small ulcer above the ankle showed vasculitis of a deep arteriole with fibrinoid changes in the wall and many mononuclear cells within and outside the vessel (Fig. 3). There was luminal narrowing, with evidence of endothelial cell proliferation on staining with Factor VIII related antigen (Fig. 4) and the internal elastic lamina was intact (Fig. 5), suggesting that the vasculitis could have been secondary to occlusion rather than primary inflammation. Mid-dermal vessels showed endothelial cell proliferation with almost complete luminal occlusion in places. There were also fibrin thrombi in some papillary blood vessels with overlying epidermal necrosis.

Anticardiolipin antibody was initially reported as absent at a time when the leg ulceration was active, but on subsequent testing, some months later in another laboratory, IgG and IgM anticardiolipin antibodies were detected in moderately positive titres by ELISA.[6] Cryoglobulins were weakly positive on some occasions but not on others. Antinuclear antibody and extractable nuclear antigens, including Ro, La, Sm and RnP, were consistently negative. Other

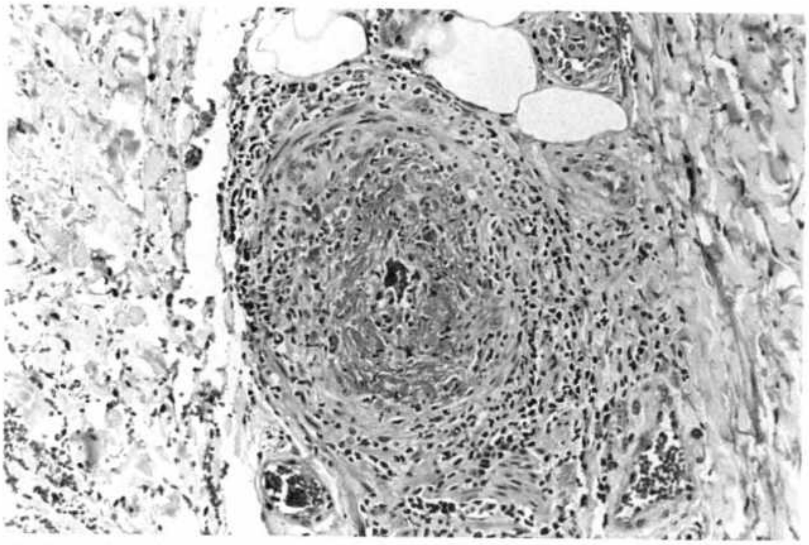

FIGURE 3. Biopsy from the ankle showing a dense mononuclear infiltrate within and outside a deep dermal arteriole, narrowing of the lumen and fibrinoid change in the wall. Haematoxylin & Eosin × 100 original magnification.

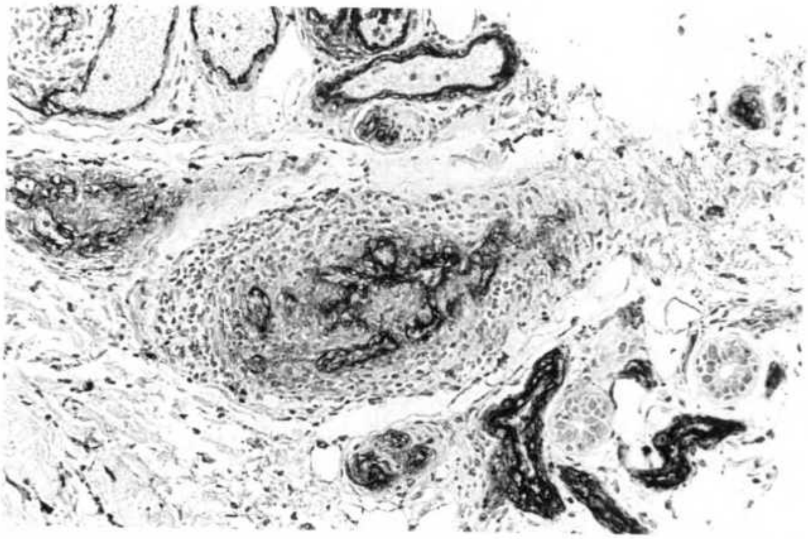

FIGURE 4. Endothelial cell proliferation demonstrated by positive staining for Fador VIII related antigen (indirect immunoperoxidase technique) × 100 original magnification.

normal or negative investigations included kaolin partial thromboplastin time, VDRL, full blood count, erythrocyte sedimentation rate and tests for circulating immune complexes. A CAT scan of her head showed slight prominence of the right occipital and frontal horns of the lateral ventricle (Fig. 6) and some degree of cortical atrophy.

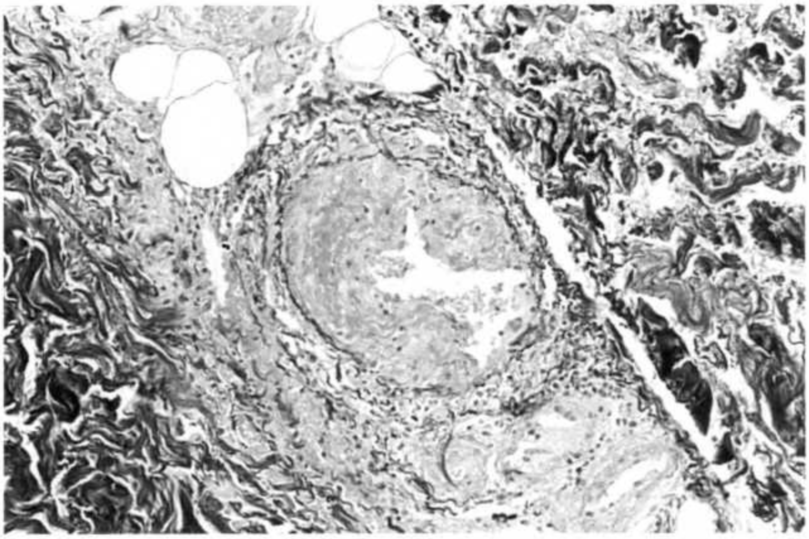

FIGURE 5. Endothelial cell proliferation within the internal elastic lamina. Elastic van Giesen × 100 original magnification.

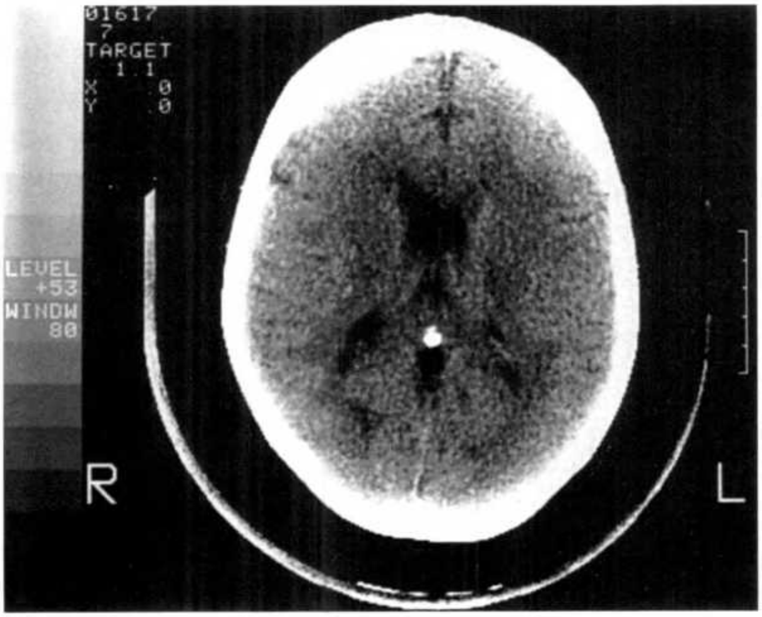

FIGURE 6. CT scan of the head (Cut 7) showing asymmetry and slight enlargement of the right lateral ventricle.

DISCUSSION

The cause of Sneddon's syndrome is not known, but a study of the cerebral and hand arteriograms in eight patients showed multiple occlusions in medium-sized arteries.[3] These patients also showed intimal hyperplasia on digital artery biopsy, with relatively little inflammation. CAT scan showed some degree of cortical atrophy. Tests for anti-nuclear factor and LE cells were negative but the anticardiolipin antibody test was apparently not performed. This test has only recently become widely available, and most other authors who have described cases of Sneddon's syndrome have not mentioned the test.[7-11] Conversely, Hughes, in his review of syndromes associated with the anticardiolipin antibody, did not mention the Sneddon syndrome.[12] Other authorities, however, now seem to have accepted that the Sneddon syndrome is likely to be associated with the presence of anticardiolipin antibodies,[13,14] and Jonas *et al.*[15] have reported a case of Sneddon's syndrome with labile hypertension and retinal artery occlusion in whom anticardiolipin antibody was present. It is interesting that in our patient the test was initially negative several weeks after the onset of the cerebral ischaemia, at a time when livedo and leg ulcers were present, but subsequently became positive when the test was repeated in a different hospital. This might, however, be attributable to differences in laboratory technique, and does not necessarily imply that the anticardiolipin antibody plays no role in the pathogenesis of the disease.

None of the patients previously described as Sneddon's syndrome seem to have had frank cutaneous ulceration. The present patient had lesions on the lower legs which fit the diagnosis of livedo vasculitis on clinical and histological grounds.[4] We have not been able to find any report of the presence or absence of anticardiolipin antibodies in this condition, although it seems likely that the antibody might have been present in patients with a similar condition described as 'summer ulceration' of the legs associated with livedo reticularis.[16,17] Some of these cases also had hypertension, Raynaud's phenomenon, acrocyanosis and thrombosis of the digital arteries, which are features of the anticardiolipin syndrome.[12-14] Johansson *et al.* have reported five patients with deep vein thrombosis and the circulating lupus anticoagulant, and their patients also had necrotizing purpura and painful superficial ulcers around the ankles.[5] Burton[18] has postulated a possible relationship between these cases with multiple shallow leg ulcers leading to the 'atrophie blanche' type of scarring and the 'cutaneous Degos' syndrome, with porcelain-white scars and an apparent absence of gastrointestinal lesions.[19] The exact nosological status of this so-called 'cutaneous Degos' syndrome, which can be mimicked by systemic lupus erythematosus,[20] is uncertain. It may be relevant, however, that the Degos' syndrome, like Sneddon's syndrome, can be associated with cerebral thrombosis[21] and with anticardiolipin antibodies,[22] and it is possible, albeit far from proven, that these various syndromes share a related pathogenesis.

The present patient fulfils all the criteria for Sneddon's syndrome, and the presence in the same patient of leg ulcers closely resembling livedo vasculitis supports the idea that these two conditions may be related. Anticardiolipin antibodies predispose to thrombosis and vascular occlusion by a mechanism which is as yet not fully understood.[13,14] Further work is required to determine the role, if any, of intimal proliferation, and defects of local coagulation, fibrinolysis and tissue plasminogen activator levels in the pathogenesis of conditions characterized by the presence of anticardiolipin antibodies. We suggest that these antibodies should be tested for routinely in patients with either the Sneddon syndrome or any of the types of leg ulceration which might be related to the livedo vasculitis spectrum. It might also be worthwhile to investigate the local coagulation systems in such patients if facilities are available, as a defect might cause small vessel occlusion with a relative sparcity of inflammatory change. It would also be of interest to perform cerebral CAT scans in patients with livedo vasculitis to look for subclinical evidence of the Sneddon syndrome.

Our patient's leg ulceration responded well to treatment with dipyridamole and aspirin, and there have been no further cerebro-vascular episodes since this treatment was started. Other authors have recently suggested that anti-platelet drugs such as ticlopidine hydrochloride, dipyridamole and low-dose aspirin are the treatment of choice for livedo vasculitis,[23,24] and our experience in this patient would support this suggestion.

REFERENCES

1 Champion RH, Rook AJ. Livedo reticularis *Proc Roy Soc Med* 1960; **53:** 961–2.
2 Sneddon IB. Cerebro-vascular lesions and livedo reticularis. *Br J Dermatol* 1965; **77:** 180–5.
3 Rebollo M, Val JF, Garijo F *et al.* Livedo reticularis and cerebrovascular lesions (Sneddon's syndrome). Clinical, radiological and pathological features in 8 cases *Brain* 1983; **106:** 965–79.
4 Bard JW, Winkelmann RK. Livedo vasculitis. Segmental hyalinising vasculitis of the dermis. *Arch Dermatol* 1967; **96:** 489–99.
5 Johansson EA, Niemi KM, Mustakallio KK. A peripheral vascular syndrome overlapping with systemic lupus erythematosus. *Dermatologica* 1977; **155:** 257–99.
6 Gharavi AE, Harris EN, Asherson RA, Hughes GRV. Anticardiolipin antibodies: isotype distribution and phospholipid specificity. *Ann Rheum Dis* 1987; **46:** 1–6.
7 Rumpl E, Rumpl H. Recurrent global amnesia in a case with cerebrovascular lesions and livedo reticularis (Sneddon's syndrome). *J Neurol* 1979, **221:** 127–31.
8 Stevens WP, Ferguson IT. Livedo reticularis and cerebro-vascular disease. *Postgrad Med J* 1982; **58:** 70–3.
9 Quimby SR, Perry HO. Livedo reticularis and cerebrovascular accidents. *J Am Acad Dermatol* 1980; **3:** 377–9.
10 Thomas DJ, Kirby JDT, Britton KE, Galton DJ Livedo reticularis and neurological lesions. *Br J Dermatol* 1982; **106:** 711–4.
11 Arnott G, Beniot M, Gosselin, *et al.* Accidents ischémiques encéphaliques successifs chez un sujet jeune. *Rev Neurol (Paris)* 1979; **135:** 221–36.
12 Hughes GRV. Connective tissue disease and the skin. *Clin Exp Dermatol* 1984; **9:** 535–9.
13 Asherson RA, Harris EN. Anticardiolipin antibodies—clinical associations. *Postgrad Med J* 1986; **62:** 1081–7.
14 Bingley PJ, Hoffbrand BI. Antiphospholipid antibody syndrome: a review *J R Soc Med* 1987; **80:** 445–8.
15 Jonas J, Kolble K, Völcker HE, Kalden JR. Central retinal artery occlusion in Sneddon's disease associated with antiphospholipid antibodies. *Am J Ophthalmol* 1986; **102:** 37–40.
16 Feldaker M, Hines EA, Kierland RR. Livedo reticularis with summer ulcerations. *Arch Dermatol* 1955; **72:** 31–3.
17 Feldaker M, Hines EA, Kierland RR. Livedo reticularis with ulcerations. *Circulation* 1956; **13:** 196–223
18 Burton JL. Livedo reticularis, porcelain-white scars, and cerebral thromboses. *Lancet* 1988; **i:** 1263–5.
19 Su WPD, Schroeter AL, Lee DA *et al.* Clinical and histologic findings in Degos' syndrome (malignant atrophic papulosis). *Cutis* 1985; **35:** 131–6.
20 Black MM, Hudson PM. Atrophie blanche lesions closely resembling malignant atrophic papulosis (Degos' disease) in systemic lupus erythematosus. *Br J Dermatol* 1976; **95:** 649–52.
21 Winkelmann RK, Howard FM, Perry HP, Miller RH. Malignant papulosis of the skin and cerebrum. A syndrome of vascular thrombosis. *Arch Dermatol* 1963; **87:** 94–7.
22 Englert HJ, Hawkes CH, Boey ML *et al.* Degos' disease. Association with anticardiolipin antibodies and the lupus anticoagulant. *Br Med J* 1984; **289:** 576.
23 Drucker CR, Duncan WC. Antiplatelet therapy in atrophie blanche and livedo vasculitis. *J Am Acad Dermatol* 1982; **7:** 359–63.
24 Yamamoto M, Danno K, Shio H, Imamura S. Antithrombotic therapy in livedo vasculitis. *J Am Acad Dermatol* 1988; **18:** 57–62.

Journal of British Dermatology (1989) **120**, 449–453.

Pemphigoid vegetans

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SUMMARY

A Japanese case of pemphigoid vegetans is described. The clinical, histopathological and immunological features were similar to the previously reported cases. The patient also developed vesicular lesions. Indirect immunoelectronmicroscopy revealed that the autoantibody in this patient's serum reacted with basal cell hemidesmosomes. This study provides further evidence that pemphigoid vegetans is a subtype of bullous pemphigoid.

Pemphigoid vegetans was first reported in 1979 by Winkelmann and Su. It has a clinical resemblance to pemphigus vegetans, but there are histological and immunopathological features of bullous pemphigoid.[1] Since then two similar cases have been reported, one from the United States[2] and one from Europe.[3] We now report another case of pemphigoid vegetans seen in a Japanese man. This patient also had vesicular lesions of pemphigoid after the clearance of the vegetating lesions. Indirect immunoelectronmicroscopic studies revealed that serum antibodies reacted to the hemidesmosomes of the basal cells.

CASE REPORT

A 75-year-old Japanese man was first seen in January 1985 with a pruritic eruption on his groin and periumblical region which had developed over the previous 2 months. Physical examination revealed well-defined pigmented, vegetating and indurated plaques on his groin and perianal area with small bullae and vesicles at their periphery (Fig. 1). There were crusted and eroded brown macule with small vesicles seen bilaterally on the upper eye-lids, lips, neck and thighs. He had some transient soreness of the tongue but no apparent lesions were seen among the oral mucosa.

Laboratory investigations were carried out and the results were as follows. The erythrocyte sedimentation rate was 28 mm/h, the leucocyte count 6,300/mm^3 (neut. 38%, eosinophil 23%, lymph. 37%), the platelet count 310,000/mm^3. Urinalysis was normal. Other blood investiga-

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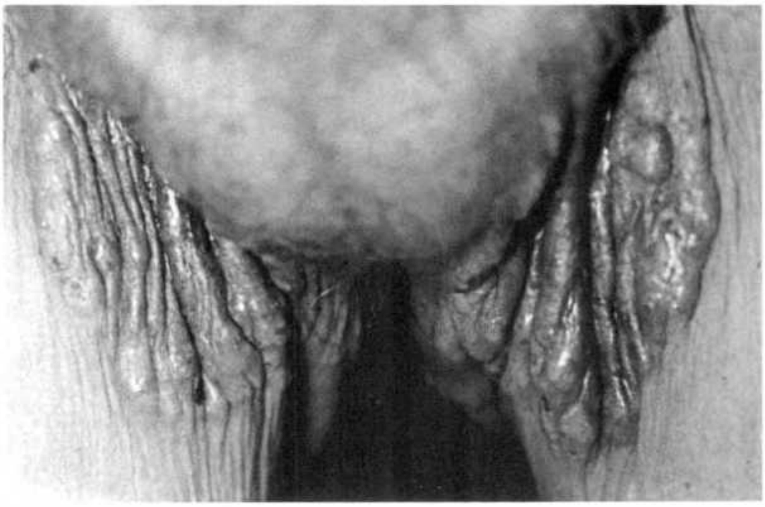

FIGURE 1. Well-demarcated, vegetating plaques with bullae or vesicles on its periphery are seen in the bilateral inguinal regions.

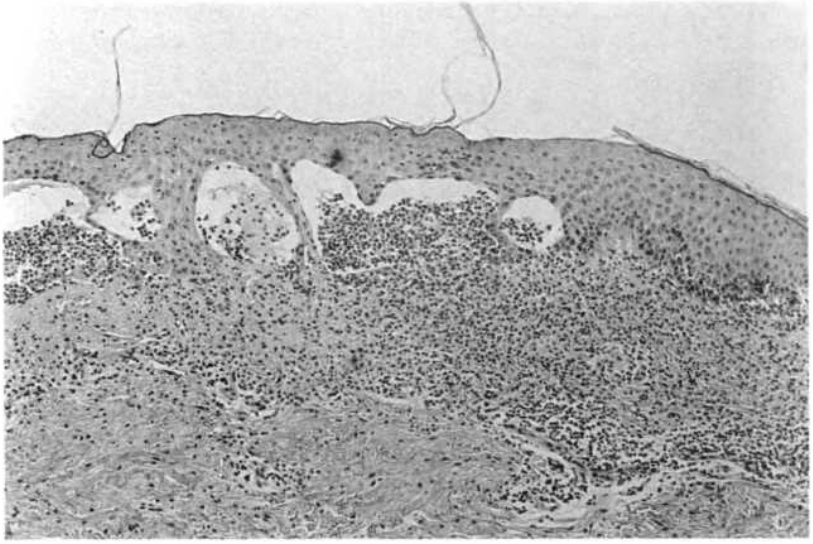

FIGURE 2. Biopsy specimen taken from the groin showing acanthosis subepidermal and blistering accompanied by eosinophil-dominant dense cell infiltration (hematoxylineosin stain; original × 200).

tions were normal. The immunoglobulin and complement levels were also normal apart from IgE, 330 IU/ml. Antinuclear antibody, antiSS-A antibody, antiSS-B antibody were all negative. The ECG and chest X-ray were normal and there was no evidence of inflammatory bowel disease either clinically or on X-ray examination.

Biopsy specimens taken from the groin showed acanthosis and subepidermal blistering with many eosinophils in the upper dermis and in the blister (Fig. 2). No acantholysis was seen. Direct immunofluorescence revealed the deposition of IgG and C3 at the basement membrane

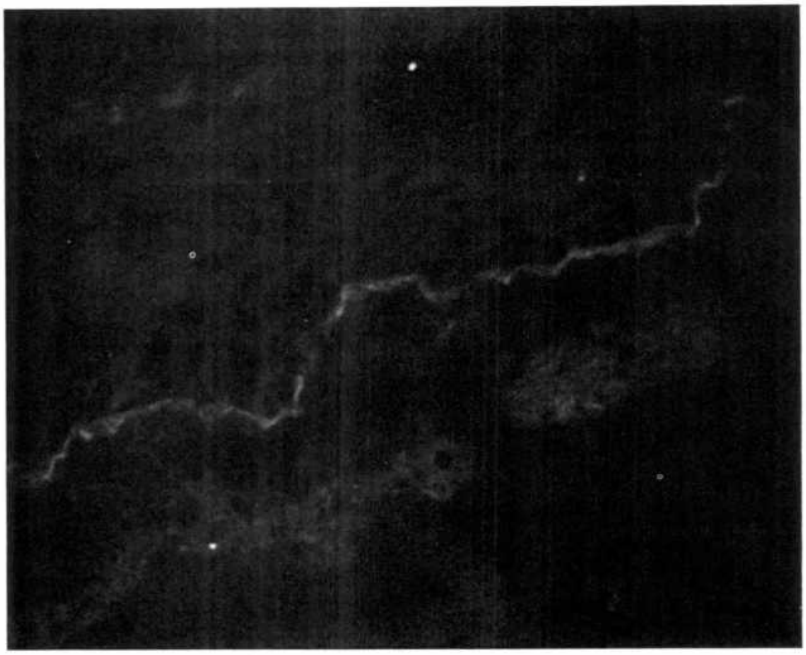

FIGURE 3. Direct immunofluorescence study showing IgG deposition at the epidermal basement membrane zone. (Original × 200).

zone of the epidermis (Fig. 3). No IgM, IgA deposits were seen. Indirect immunofluorescence showed positive antibasement membrane antibody of IgG class at a titre of 1:10,240 (Fig. 4). Indirect immunoelectron microscopic study was performed as previously described.[4] The reaction product was in the region of the basal cell hemidesmosomes (Fig. 5). A diagnosis of

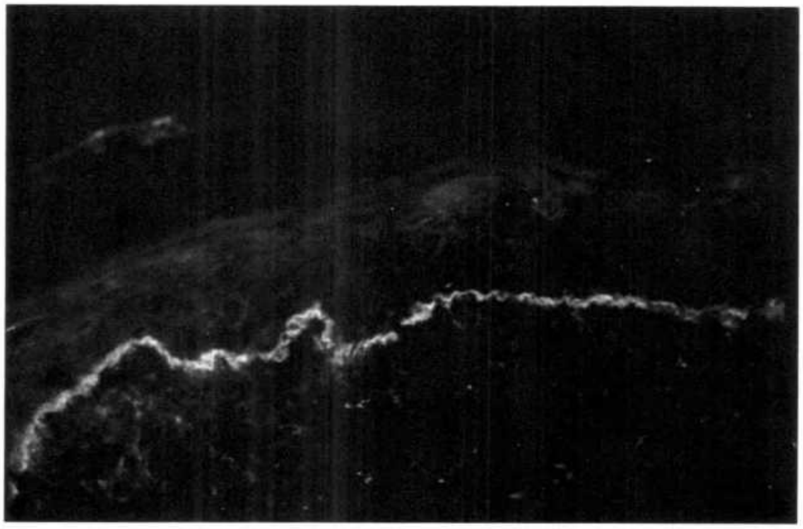

FIGURE 4. Indirect immunofluorescence study demonstrating antibasement membrane antibody of IgG class in the serum of our patient. (Original × 200).

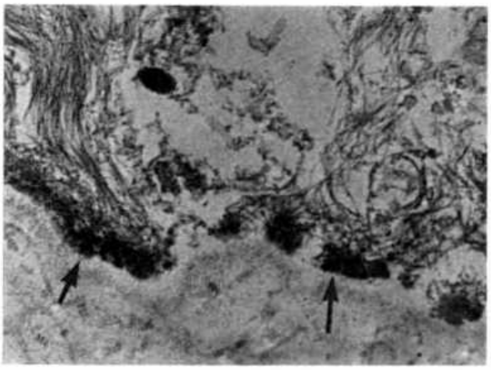

FIGURE 5. Indirect immunoelectron microscopic study showing the reaction products localize at the basal cell hemidesmosomes (arrow). (Original × 24,000)

pemphigoid vegetans was made and he was treated with topical application of betamethasone valerate. There was a remarkable improvement, and all lesions cleared within 3 weeks leaving residual brown stains.

A few weeks after the clearance of the vegetating lesions a new eruption appeared on his forearm, with many small tense vesicles set in areas of erythemas and accompanied by severe itching (Fig. 6). A biopsy specimen showed subepidermal blistering with an infiltrate of eosinophils. Immunofluorescent studies revealed IgG and C3 deposits, but not IgM or IgA at the basement membrane zone. We diagnosed this condition as vesicular pemphigoid and topical application of betamethasone valerate was again effective as treatment. Antibasement membrane antibody remained high (titre 1:2,560), with eosinophils persisting as 15% of the total white cell count in peripheral blood even after the eruptions had disappeared.

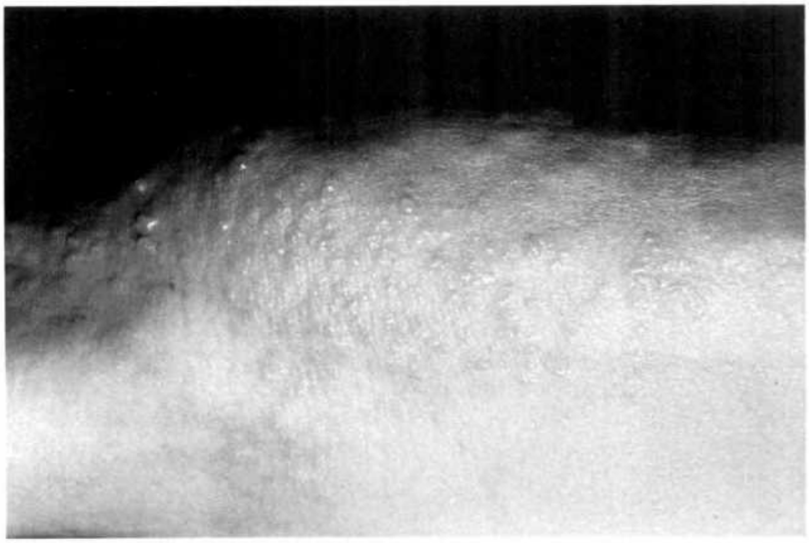

FIGURE 6. A lot of tiny, tense vesicles with erythemas are seen on his forarm, diagnosed as vesicular pemphigoid.

DISCUSSION

Pemphigoid vegetans was first described by Winkelmann and Su in 1979[1] but two other cases have subsequently been reported.[2,3] The clinical features of the disease are circumscribed, hypertrophic lesions with crust or erosion; pustules or vesicles at the periphery, affecting mainly the intertriginous region, resemble pemphigus vegetans. Histopathologically, there are subepidermal clefts with eosinophil infiltration and acanthosis of the epidermis but no acantholysis. Immunofluorescent studies have revealed the *in vivo* deposition of IgG and C3 at the dermo-epidermal junction. Circulating antibodies of IgG class against epidermal basement membrane zone have been detected. The condition is considered to be a subtype of bullous pemphigoid. Our case had almost all the clinical, histopathological and immunopathological features of pemphigoid vegetans. Our patient also developed another variant of bullous vesicular pemphigoid,[5,6] on the disappearance of the vegetating lesions.

Recently, Wolff *et al.*[7] reported a case presenting a similar condition of pemphigoid vegetans with scarring of mucous membrane and which resembled cicatricial pemphigoid. They called it vegetating cicatricial pemphigoid and regarded it as a new subset of cicatricial pemphigoid. Kuokkanen *et al.*[2] noted scarring in their case but the relationship of this scarring condition and pemphigoid vegetans is not known.

It has been reported that immunoelectronmicroscopic studies have revealed that bullous pemphigoid antigens localize at basal cell hemidesmosomes and in the upper part of lamina lucida. In cicatricial pemphigoid, however, the antigens localize at the lower part of the lamina lucida.[8–10] Immunoelectronmicroscopic study has not been performed on either pemphigoid vegetans or vegetating cicatricial pemphigoid. Indirect immunoelectronmicroscopic studies in our patient showed that the reaction product was localized at the hemidesmosomes of the basal cells, as seen in bullous pemphigoid.[8] Thus, our results further support the concept that pemphigoid vegetans is a subtype of bullous pemphigoid.

REFERENCES

1 Winkelmann RK, Su WPD. Pemphigoid Vegetans. *Arch Dermatol* 1979; **115**: 446–48.
2 Kuokkanen K, Helin H. Pemphigoid Vegetans. *Arch Dermatol* 1981; **117**: 56–7.
3 Al-Najjar A, Reilly GD, Bleehen SS. Pemphigoid vegetans: A case report. *Acta Derm Venereol (Stockh)* 1984; **64**: 450–52.
4 Matsukawa A, Furue M, Yu H *et al.* Epidermolysis bullosa acquistia diagnosed by immunoelectronmicroscopy. *J Dermatol (Tokyo)* 1983; **10**: 347–53.
5 Bean SF, Michel B, Furey N *et al.* Vesicular pemphigoid. *Arch Dermatol* 1976; **112**: 1402–404.
6 Gruber GG, Owen LG, Callen JP. Vesicular pemphigoid. *J Am Acad Dermatol* 1980; **3**: 619–22.
7 Wolff K, Rappersberger K, Steiner A, Konrad K. Vegetating cicatricial pemphigoid: A new subset of the cicatricial pemphigoid spectrum. *Arch Dermatol Res* 1987; **279**: (Suppl) 30–37.
8 Westgate GE, Weaver AC, Conchman JR. Bullous pemphigoid antigen localization suggests an intracellular association with hemidesmosomes. *J Invest Dermatol* 1985; **84**: 218–24.
9 Fine JD, Neises GR, Katz SI. Immunofluorescence and immunoelectron microscopic studies in cicatricial pemphigoid. *J Invest Dermatol* 1984; **82**: 39–43.
10 Katz SI. The epidermal basement membrane zone: Structure, ontogeny, and role in disease. *J Am Acad Dermatol* 1984; **11**: 1025–37.

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Erythema multiforme due to griseofulvin

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SUMMARY

Three patients are reported who developed erythema multiforme during treatment with griseofulvin. To our knowledge there have been no previously documented reports of this drug causing erythema multiforme in either the English or American literature.

Erythema multiforme is a distinctive clinical and histological reaction to many different stimuli. The most thoroughly documented associations are with infections of herpes simplex and *Mycoplasma,* although in about half the cases no precipitating factor may be established.[1] Exposure to drugs may also precipitate erythema multiforme but evidence of a direct link between administration of the drug and precipitation of the cutaneous eruption is often lacking. It is probably for this reason that some drugs are readily accepted as causing erythema multiforme,[2,3] e.g. barbiturates, sulphonamides and the drugs chemically related to sulphonamides, whereas other drugs such as griseofulvin are listed but appear unreferenced in texts.[4,5] We wish to describe three patients who developed erythema multiforme during treatment with griseofulvin and in whom we consider the griseofulvin was the aetiological factor. A proven causal relationship was not established because it was not considered ethical to expose the patients to further risk by rechallenge with the drug.

CASE REPORTS

Patient 1

A 29-year-old bisexual male art dealer was found to be HIV 1 positive in December 1986. He attended the Department of Dermatology in June 1988 for treatment of a scaly eruption, involving several of the toe web spaces and the plantar surfaces of both feet, which had been present for 4 months. He also had recently developed crumbling and thickening of all the toe nails. His general health was good and he denied taking any medications. On examination there were scaly annular plaques with central clearing on the limbs, onychomycosis of all toe nails and tinea pedis of the toe web spaces and the plantar surfaces of both feet. Mycology of specimens from each site grew *Trichophyton rubrum*. The patient was started on griseofulvin at a daily dose of 1 g. Three days later he developed a burning, annular, erythematous eruption with iris lesions

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involving the distal limbs and the palmar and plantar surfaces. A diagnosis of erythema multiforme was made, the griseofulvin was stopped and the eruption gradually resolved over 2 weeks.

Patient 2

A 21-year-old male homosexual actor was found to be HIV 1 positive in May 1987. In January 1988 he developed a scaly rash on the sides of his nose, in the scalp and over both elbows. At the same time he also noticed that several finger and toe nails had become thickened and crumbly. In the past he had suffered from mild atopic eczema and hayfever and there was a family history of atopic eczema. His general health was otherwise good and he was not taking any drugs. On examination in June 1988 there was oral candidiasis, seborrhoeic dermatitis involving the nasolabial folds and the scalp, plaques of psoriasis over both elbows and onychomycosis with onycholysis affecting three finger nails on the right hand and all toes on the right foot. Finger and toe-nail clippings grew *T. rubrum*. The seborrhoeic dermatitis and psoriasis were treated with a moderately potent topical corticosteroid preparation and the patient was started on griseofulvin 500 mg b.i.d. Nine days after starting the griseofulvin, the patient developed an erythematous maculo-papular eruption with target lesions on the face, trunk and acral surfaces of both arms and legs. The griseofulvin was stopped and the eruption resolved after 17 days.

Patient 3

A 29-year-old woman attended with several non-itchy, scaly, annular erythematous lesions on her buttocks, knees and thighs. A diagnosis of tinea corporis was made after direct microscopy of skin scrapings showed fungal hyphae. She was treated with topical miconazole cream and although there was a modest improvement in some lesions, new ones continued to appear. She was, therefore, prescribed griseofulvin 1 g daily. Ten days later she developed a florid rash on her face, arms, trunk and legs. On examination there were multiple well demarcated erythematous maculo-papular lesions 1–2 cm in diameter together with several target lesions on the limbs and trunk. The appearance was characteristic of erythema multiforme. On direct questioning the patient denied taking any other medications and apart from the initial skin eruption had not developed any new symptoms. In view of the widespread distribution of the erythema multiforme the patient was treated with systemic steroids and within 2 weeks the eruption had resolved completely.

A skin biopsy from a lesion on the back showed a perivascular lymphocytic infiltrate in the dermis with extravasation of red blood cells. Direct immunofluorescence was negative. A competitive monoclonal ELISA test for anti-HIV 1 was negative.

DISCUSSION

Data extracted from the Committee on Safety of Medicines records over the period July 1963 to November 1987 show that of the total of 383 reports of adverse reactions to griseofulvin, there were 166 reactions affecting the skin and these included 10 cases of erythema multiforme and 3 cases of Stevens–Johnson syndrome (Committee on Safety of Medicines, personal communication). However, following an extensive search of the literature we have been able to find only two reports of griseofulvin causing erythema multiforme, one each in the Russian[6] and Dutch literature[7] and none in the English or American literature.

It is possible that the development of the erythema multiforme in our patients was not as a result of the griseofulvin therapy. Dermatophyte infections are included in the list of causes of erythema multiforme,[8] but this association is poorly established and all three patients had suffered from the dermatophyte infections for several months before the onset of the erythema multiforme. Consequently, we do not consider that the dermatophyte infections were responsible for the aetiology of the erythema multiforme in our patients. Furthermore, the 3- and 10-day interval between administration of griseofulvin and the development of the skin lesions supports the possibility of a hypersensitivity reaction to the drug.[9]

The first two patients who developed erythema multiforme were both HIV positive. We considered the notion that patients infected with HIV may be unusually sensitive to griseofulvin and that we were observing a phenomenon similar to that which occurs in patients with infectious mononucleosis treated with ampicillin. However, the third patient was HIV negative and there have been no reports of erythema multiforme developing after treatment with griseofulvin in America, where there are many more HIV-positive patients.

Griseofulvin is a frequently used drug in Great Britain with approximately 180,000 prescriptions dispensed by retail chemists during the years 1986 and 1987 (DHSS Statistics and Research Division). Side-effects and adverse reactions to griseofulvin are uncommon and are usually mild and transient. The drug may cause photosensitivity,[10] pruritus,[11] urticaria,[12,13] cold urticaria,[14] fixed drug eruptions,[15,16] angio-oedema,[17] serum sickness,[18] exanthematic eruptions[19] and exfoliative dermatitis.[20] Griseofulvin may also precipitate or exacerbate systemic lupus erythematosus and there has been a report of a fatal outcome.[21] Griseofulvin also can disturb porphyrin metabolism by inducing hepatic δ-aminolevulinic acid synthetase and inhibiting ferrochelatase activity.[22] Thus, although griseofulvin has little effect on porphyria cutanea tarda, attacks of acute intermittent porphyria and variegate porphyria may be precipitated.

The cause of erythema multiforme is frequently not determined. We wish to add griseofulvin to the list of drugs which may induce this eruption. These three cases have been reported to the Committee on Safety of Medicines.

REFERENCES

1 Champion RH. Erythema multiforme. In *Textbook of Dermatology* (Rook AJ, Wilkinson DS, Ebling FJ *et al.*, eds), 4th edn., Vol 2. Oxford: Blackwell Scientific Publications, 1986; 1085–8.

2 *Textbook of Adverse Reactions.* (Davies PM, ed), 3rd edn., Oxford: Oxford University Press, 1985; 475–6.

3 Azinge NO, Garrick GA. Stevens–Johnson syndrome (erythema multiforme) following ingestion of trimethoprim-sulfamethoxazole on two separate occasions in the same person. *J Allergy Clin Immunol* 1978; **62:** 125–6.

4 Wintroub BU, Stern RS, Arndt KA. Cutaneous reactions to drugs. In· *Dermatology in General Medicine* (Fitzpatrick TB, Eisen AZ, Wolff K *et al*, eds), 3rd edn., Vol 1. New York· McGraw-Hill, 1987; 1353–66

5 Becker LE. Griseofulvin. *Dermatol Clin* 1984; **2:** 115–20.

6 Walinga H, van Beugen L. Syndroom van Stevens–Johnson na gebruik van griseofulvine. *Ned Tijdschr Geneeskd* 1981; **125:** 759–60.

7 Belkin BG & Saiyan ESh. On the etiology and pathogenesis of Stevens–Johnson syndrome. *Vestn Dermatol Venerol* 1973; **47:** 61–4.

8 Elias PM, Fritsch PO. Erythema multiforme. In: *Dermatology in General Medicine* (Fitzpatrick TB, Eisen AZ, Wolff K *et al.* eds), 3rd edn., Vol 1. New York· McGraw-Hill, 1987; 555–63.

9 Kazmierowski JA, Wuepper KD. Erythema multiforme. In: *Pathogenesis of Skin Disease* (Thiers BH, Dobson RL, eds), New York: Churchill Livingstone, 1986; 305–16

10 Jarratt M. Drug photosensitization. *Int J Dermatol* 1976; **15:** 317–23.

11 Quero R. Allergic reaction to griseofulvin. *J Invest Dermatol* 1960; **34:** 283–4.

12 Blank H, Roth FJ. The treatment of dermatomycoses with oraly administered griseofulvin. *Arch Dermatol* 1959; **79:** 259–66.

13 Feinstein A, Sofer E, Trau H, Schewach-Millet M. Urticaria and fixed drug eruption in a patient treated with griseofulvin. *J Am Acad Dermatol* 1984; **10:** 915–7.

14 Chang T. Cold urticaria and photosensitivity due to griseofulvin. *J Am Med Assoc* 1965, **193:** 848–50.

15 Savage J. Fixed drug eruption to griseofulvin. *Br J Dermatol* 1977; **97:** 107–8.
16 Thyagarajan K, Kamalam A, Thambiah AS. Fixed drug eruption to griseofulvin. *Mykosen* 1981, **24:** 482–4.
17 Goldblatt S. Severe reaction to griseofulvin. *Arch Dermatol* 1961; **83:** 936–7.
18 Prazak G, Ferguson JS, Comer JE, McNeil BS. Treatment of tinea pedis with griseofulvin. *Arch Dermatol* 1960; **81:** 821–6.
19 Von Pohler H, Michalski H. Allergisches exanthem nach griseofulvin. *Dermatol Monatschr* 1972; **158:** 383–90.
20 Reaves LE. Exfoliative dermatitis occurring in a patient treated with griseofulvin. *J Amer Geriat Soc* 1964; **12:** 889–92.
21 Madhok R, Zoma A, Capell H. Fatal exacerbation of systemic lupus erythematosus after treatment with griseofulvin. *Br Med J* 1985; **291:** 249–50.
22 Bickers DR. Environmental and drug factors in hepatic porphyria. *Acta Derm Venereol (Stockh)* 1982; Suppl **100:** 29–41.

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An unusual case of trimethylaminuria

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SUMMARY

A case is reported of trimethylaminuria that first developed in adulthood without any apparent cause. The patient developed a characteristic fish odour of his sweat, urine and to his breath after the consumption of choline-rich foods. Elevated levels of trimethylamine were present in the urine after dietary tests and identified by means of gas chromatography.

Trimethylaminuria is an uncommon condition in which there in a distinctive fish odour of sweat and urine. This condition is caused by a deficiency of a liver enzyme, trimethylamine-oxidase (TMA-oxidase) and this results in accumulation of trimethylamine (TMA). TMA is produced in the gut mainly by bacterial degradation of choline-rich foods, particularly from marine animals and it is TMA, a volatile substance, that is responsible for the offensive smell of spoilt fish. This compound is converted by TMA-oxidase into a stable, non-odourous trimethylamine-N-oxide (TMA-oxide) and which is then excreted in the urine (Fig. 1). In the presence of a defective TMA-oxidase activity, the accumulated TMA is eliminated as a volatile product through the urine, sweat and breath giving the affected individual a characteristic fish smell.[1-7]

CASE REPORT

A 32-year-old man was referred to us because of a body odour problem and because, for the past 5 years, he had found that after eating fish or shellfish his body had an offensive smell of rotten fish. This smell was enhanced with increased temperature and emotion and also after physical exercise. The smell would persist for about 24 h. He was suspected of having trimethylaminuria and dietary tests were undertaken to confirm this diagnosis.

During a 10-week period of clinical and laboratory investigations, the patient was asked to eat various fish, shellfish and other choline-rich foods, e.g. eggs, liver and leguminous vegetables. In these tests and within 3 h after the meals, an unpleasant fishy odour was smelt on the skin, particularly on the palms and trunk, and the odour was also apparent in his breath. The smell was greatest after eating cod and lobster but was not noticeable after having trout. The intensity of the smell could be reduced by administering a digestive enzyme-complex, Digestopan^{R} (25

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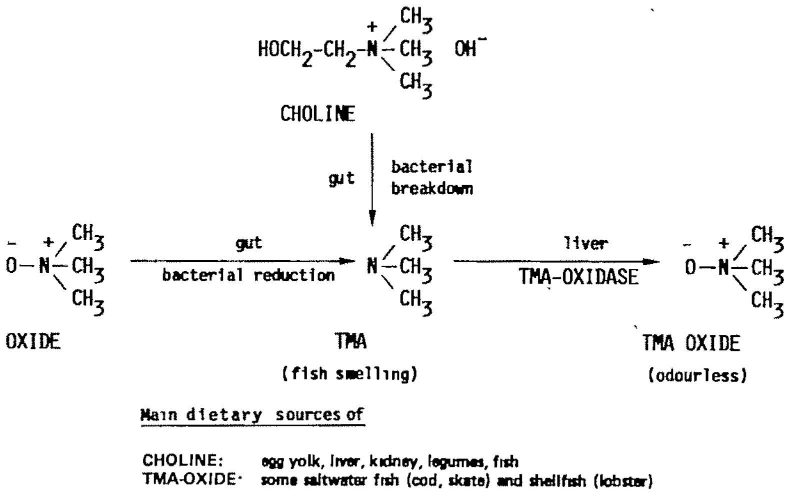

FIGURE 1. Dietary origin and metabolism of trimethylamine (TMA) in man.

mg pepsin, 50 mg papain, 15 mg diastase, 15 mg pancreatin, 13 mg pancreatic lipase, 15 mg cellulase) or acidifying the diet with yoghurt and citrus fruits.

Similar dietary tests were undertaken on the patient's family, including his mother, son, two brothers and one sister and these were all negative.

Laboratory investigations carried out on the patient were all normal and there was no evidence of any hepatic dysfunction. However, IgG antibodies against hepatitis A virus (HAVAb) were detected in the patient's serum.

The levels of trimethylamine (TMA) were assayed in the urine by gas chromatography. Aliquots of early morning and freshly voided urine were acidified to pH 2 with 6 M HCl and subsequently analysed. On a fish-free and low choline diet, the patient did not have a fishy odour

TABLE 1. Patient's sudoriferous and urinary excretion of trimethylamine (TMA) 8 h after dietary tests

Dietary test (standard edible amount: 250 g)	Odour intensity (sweat)	μg TMA/mg creatinine* (urine)
Fish and shellfish		
1 cod	+ + +	225·1
2 sole	+ +	82·4
3 grey mullet	+ +	95·7
4 cuttle-fish	+	36·7
5 lobster	+ + +	196·3
6 trout	–	4·4
Choline-rich foods		
7 egg yolk (100 g) and peas (150 g)	+	58·4
8 calf liver (150 g) and kidney (100 g)	+ +	66·3

* In normal subjects the urinary excretion of TMA is < 5 μg/mg creatinine.

and the urinary excretion of TMA was 3·7 μg/mg of creatinine, which was well within the normal range.[5] After dietary intake of marine fish and with other choline-rich food there was a marked increase in the concentration of TMA in the urine. This correlated well with the intensity of the fish odour of the patient's sweat (Table 1). Gas chromatography investigations on the expired air and on the sweat gave unreliable results and this was presumed to be due to the volatility of the TMA.

DISCUSSION

The unmistakable 'fishy' odour of this patient's sweat, urine and breath, together with the elevated concentrations of TMA after a choline-rich diet enabled us to diagnose trimethylaminuria. Compared to previously reported cases,[1-7] our patient shows two unusual features. Firstly, it occurred in an adult without any previous history in childhood and secondly, there was a very sharp correlation between the unpleasant fish odour and eating certain marine foods.

It is possible that our patient had an asymptomatic viral hepatitis evidenced by HAVAb in his serum and that there was a defect of liver enzymes and acute hepatic dysfunction. The marked fishy odour after eating cod and lobster was due to their high content of TMA-oxide[1] which is found in marine animals and which is an immediate precursor of TMA.[1,3] Choline-containing foods such as other fish, eggs, liver and leguminous vegetables release TMA via a more complex and slower metabolic path (Fig. 1). Freshwater fish are lacking in TMA-oxide[1] and eating trout did not give our patient a fishy odour. There was some improvement when digestive enzyme supplements were taken and the diet acidified. Trials using neomycin[4] and metronidazole hydrochloride[7] have also been of help in previously reported cases.

Although the fish-odour syndrome is thought to be a rare disorder, it is probably not all that uncommon, as noted in a recent random population study.[8]

As regards nomenclature, we consider that the commonly used term trimethylaminuria is too restrictive because the urine is not the only body fluid that contains TMA. In our opinion it is more appropriate for these patients to be described as having the 'fish-odour syndrome'.

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REFERENCES

1. Shewan JM. The chemistry and metabolism of nitrogenous extractives in fish. *Biochem Soc Symp* 1951; **6:** 28–48.
2. Humbert JR, Hammond KB, Hathaway WE *et al.* Trimethylaminuria: the fish-odour syndrome. *Lancet* 1970; **ii:** 770–1.
3. Lee CWG, Yu SJ, Turner BB, Murray KE. Trimethylaminuria: fishy odours in children. *N Engl J Med* 1976; **295:** 937–8.
4. Danks DM, Hammond J, Schlesinger P *et al.* Trimethylaminuria: diet does not always control the fishy odour. *N Engl J Med* 1976; **295:** 962.
5. Marks R, Greaves MW, Prottey C, Hartop PJ. Trimethylaminuria: the use of choline as an aid to diagnosis. *Br J Dermatol* 1977; **96:** 399–402.
6. Brewster M, Schedewie H. Rapid identification of trimethylaminuria as a cause of body odour.*Clin Chem* 1980; **26:** 1011.
7. Shelley ED, Shelley WB. The fish odour syndrome. Trimethylaminuria. *JAMA* 1984; **251:** 253–5.
8. Al-Waiz M, Ayesh R, Mitchell SC *et al.* Trimethylaminuria (fish-odour syndrome): an inborn error of oxidative metabolism. *Lancet* 1987; **i:** 634–5.

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A family with Dowling Degos disease showing features of Kitamura's reticulate acropigmentation

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SUMMARY

We report a family showing a combination of features described in the two, rare genodermatoses, Dowling Degos disease and Kitamura's acropigmentatio reticularis.

Dowling Degos disease is a rare genodermatosis comprising reticulate pigmentation of the flexures, prominent comedo-like lesions and pitted scars. It has been suggested[1] that this may be the same disease as Kitamura's acropigmentatio reticularis, a disorder in which acral reticulate pigmentation is the prominent feature, and in which palmar pitting with breaks in the epidermal ridge pattern over the finger tips occur. We report a family showing the features of both diseases.

CASE REPORTS

Case One

A 37-year-old male patient was first noticed to have unusual pigmentation on the dorsal aspects of his hands at the age of 7 (Fig. 1). This subsequently involved the flexor aspects of the arms and wrists, the dorsa of the feet and ankles, the medial aspects of the knees, the inner aspects of the thighs, the neck and chest. The axillae and groins were relatively spared. In all areas the pigmentation has gradually increased in intensity since it first appeared and in some areas has become confluent. It appears to be intensified by exposure to the sun.

He has also developed a large number of comedones, some up to 4 mm in diameter, over the trunk, neck, face and penis. Pitted follicular scars are present in the same distribution (Fig. 2). In addition to these lesions he gives a history of several boils and cystic lesions having been excised from different sites over the years. Morton's neuromas have been excised from under both middle toe metatarso-phalangeal joints. In his hands there are numerous small palmar pits and breaks in the epidermal ridge pattern over the finger tips.

He has two sons aged 9 and 6 and a brother who also has two children aged 10 and 13 who are unaffected.

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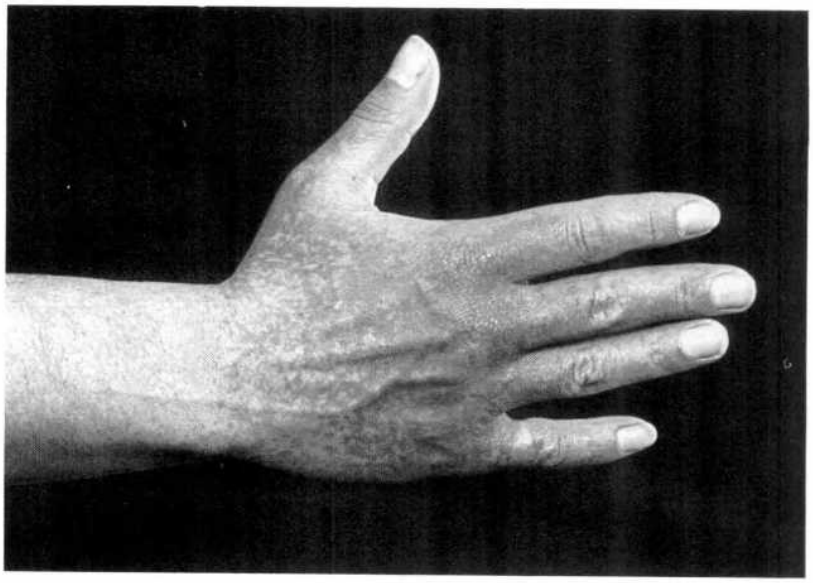

FIGURE 1. Case 1. Reticulate and confluent pigmentation over the dorsum of the right hand.

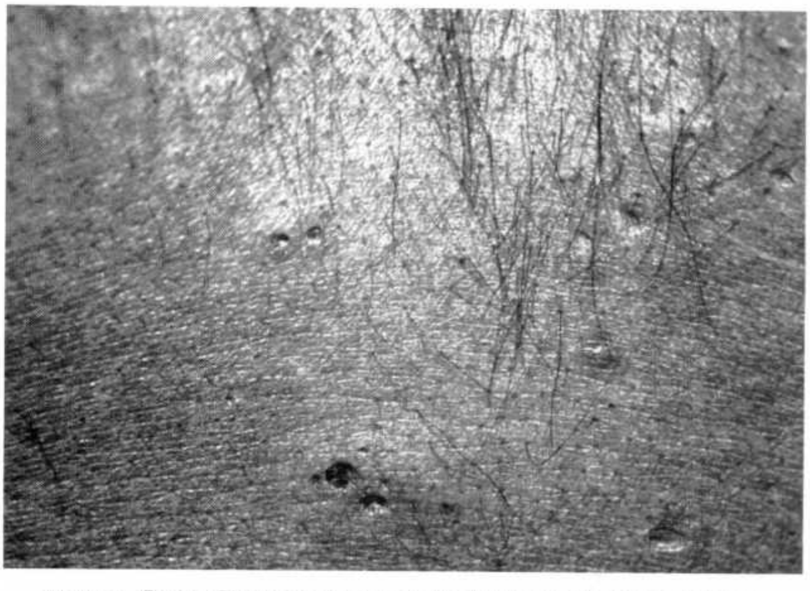

FIGURE 2. Case 1. Giant comedones and pitted scars over the upper abdomen.

Case 2

The mother of our first patient, aged 70, was also examined. She had hyperpigmentation in a distribution identical to that of her son and which appeared at the age of 14. It has progressively increased in intensity. The pigmentation is now substantially darker than her son's. She had increased pigmentation under the bra-strap areas. There were numerous pitted scars around her neck, her finger tips showed breaks in the epidermal ridge pattern, and she had palmar pitting.

She has also had numerous cysts excised from her neck and has two lipomas symmetrically situated on the flexor aspect of the middle of each forearm.

Her brother (whom we have not been able to examine), apparently has reticulate pigmentation in an identical distribution, but her sister is unaffected. There is, unfortunately, very little information about her parents who both died at a young age in Poland during World War II. An interesting point is that both our first case and his mother are dark haired individuals, whereas his uncle has fair hair and we understand that the pigmentation is less pronounced in this uncle, suggesting that the expression of the disease may be to some extent dependant on the intensity of background pigmentation.

Histology from an involved area of the forearm of case 1 showed numerous digitate projections of epidermis into the dermis which, in places, showed a tendency to branch (Fig. 3). These were markedly pigmented, especially towards the tip of each downgrowth. There was no epidermal atrophy.

DISCUSSION

Dowling Degos disease (DDD), or reticulate pigmented anomaly of the flexures, was first characterized by Wilson Jones and Grice in 1978 in their description of ten patients with the disorder.[2] The first descriptions are attributed to Dowling and Freudenthal in 1938[3] and Degos and Ossipowski in 1954.[4] Inheritance appears to follow an autosomal dominant pattern.[5] Onset is most commonly in adult life, but may occur in childhood. The typical features are reticulate pigmentation of the flexures, comedones, also known as dark dot follicles, and pitted scars.

The pigmentation in DDD affects principally the axillae, groins, sub-mammary folds and neck, sometimes extending to the face, chest, perineum, natal cleft, and wrists. It is exacerbated by sunlight and sometimes appears more prominent in the bra-strap area suggesting that friction or pressure may increase the pigmentation.

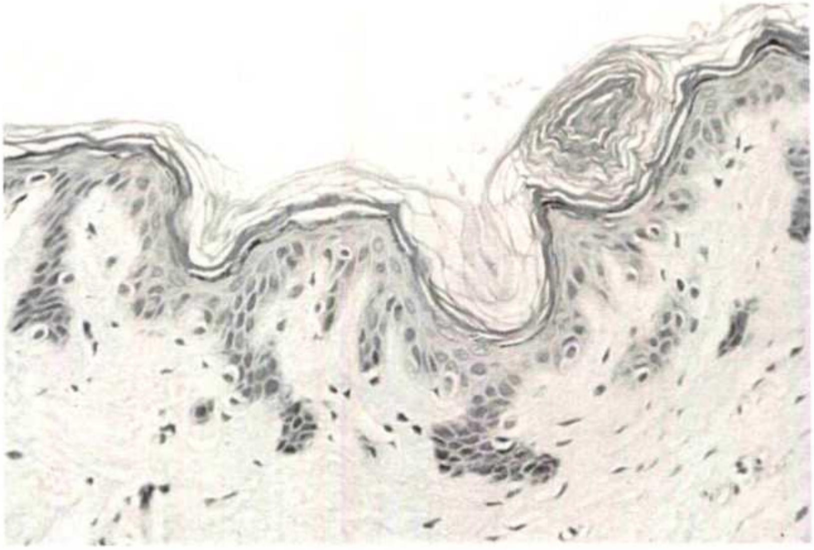

FIGURE 3. Histology from the left forearm of case 1. Epidermal downgrowths are seen which tend to branch and are markedly pigmented at the tips. (Original × 200).

Comedones and pitted scars develop most characteristically around the lateral margins of the mouth, frequently involving other areas of the face and neck and less often the axillae, thighs, abdomen and ante-cubital fossae. In addition to comedones, inflamed cystic lesions and abscesses are described involving the trunk and axillae.[2,5]

Kitamura's acropigmentatio reticularis (KAR) was first described in the Japanese literature by Kitamura and Akamatsu in 1943,[6] and in other races by Griffiths in 1976.[7] This condition is, like DDD, inherited in an autosomal dominant pattern and usually begins in childhood. Reticulate pigmentation tends to begin peripherally involving initially the dorsa of the hands and feet, and spread proximally to involve the arms, legs and less often the face, neck and trunk. The pigmented areas tend to be slightly depressed below the level of the unaffected skin. Pits or breaks in the epidermal ridge pattern of the palms, fingers and soles are often present. Dark dot follicles are not a feature.

Histologically, both DDD and KAR demonstrate epidermal downgrowths into the dermis which contain a large quantity of melanin.[2,8] One distinguishing feature is the presence of epidermal atrophy in KAR which has not been reported in DDD.

Crovato *et al.*[1] described a patient having both distal and flexural pigmentation, with some breaks in the epidermal ridge pattern of the palms. They reported mild histological atrophy of the epidermis from an involved area of the hand. This patient's family contained a member with pigmentation typical of DDD and another with pigmentation typical of KAR, and the authors therefore proposed that these two diseases might be one and the same. However, unlike our patients, none of the family they described had dark dot follicles or pitted scars.

Our family also appears to have features which have been reported in both diseases, but the features are more consistent in the family members affected than in the pedigree reported by Crovato. They have the distal reticulate pigmentation, palmar pits and breaks in the epidermal ridge pattern of KAR, and also the flexural pigmentation, dark dot follicles and pitted scars of DDD. The histology from the forearm of our patient is compatible with both diseases, but does not show the epidermal atrophy often seen in KAR.

We consider that this case provides further evidence in support of the proposal that DDD and KAR are each part of a spectrum of the same genodermatosis.

REFERENCES

1 Crovato F, Desirello G, Rebora A. Is Dowling Degos disease the same disease as Kitamura's reticulate acropigmentation? *Br J Dermatol* 1983, **109**: 105–10.

2 Wilson Jones E, Grice K. Reticulate pigmented anomaly of the flexures. *Arch Dermatol* 1978; **114**: 1150–7.

3 Dowling GB, Freudenthal W. Acanthosis nigricans. *Br J Dermatol* 1938; **50**: 467–71.

4 Degos R, Ossipowski B. Dermatose pigmentaire réticulée des plis. *Ann Dermatol Syphilol* 1954; **81**: 147–51.

5 Brown W. Reticulate pigmented anomaly of the flexures. *Arch Dermatol* 1982; **118**: 490–3.

6 Kitamura K, Akamatsu S. *Rinsho no Hifu Hitsunyo* 1943; **8**: 201. (Quoted by Griffiths, 1976)

7 Griffiths WAD. Reticulate acropigmentation of Kitamura. *Br J Dermatol* 1976; **95**: 437–43.

8 Kitamura K, Akamatsu S, Hirokawa K. Eine besondere form der acropigmentation: Acropigmentatio reticularis. *Hautarzt* 1953; **4**: 152–56.

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Obituary

Dr H.W.Gordon, M.C., M.B., F.R.C.P.

Dr Hugh Gordon died in Scotland on 11 December 1987. He had been born 90 years earlier in Maxwelltown, Kirkcudbrightshire. For the last 20 years he had lived happily there, near to his son, enjoying golf and bridge in the company of his family and grandchildren. He had practised as a dermatologist in London for over 40 years having been on the staff of St George's, the Royal Marsden and the West London Hospitals.

Hugh Walker Gordon was the elder son of H. Sharpe Gordon, one of a family of lawyers and who had been successfully involved in business in the Far East. He was educated at Marlborough but towards the end of the First World War found himself in the army as a Subaltern in the Royal Field Artillery. He was wounded, decorated with the Military Cross and invalided out. Later he was to gain a History Exhibition to Pembroke College, Cambridge and it was there that his thoughts turned to medicine, so, after completing his second M.B. he won a Scholarship to St George's Hospital Medical School for his clinical studies.

It was a small school, most of the students having been to Cambridge and it had a somewhat select, in the social sense, atmosphere. Gordon found himself with several of his Cambridge and ex-service contemporaries, indeed a number of them were later to find themselves on the consulting staff. After the usual house jobs he became R.M.O., a much prized post, often leading to a Staff appointment. These were the pre-N.H.S. days when all consultants were honorary, giving the Hospital their time free and earning their living from private practise. St George's at Hyde Park Corner, being so geographically situated, was an admirable centre from which to work.

Hugh Gordon was as charming as he was handsome, a tall, erect military figure with a somewhat authoritarian voice. He could not fail to be a favoured candidate for success and a staff appointment. At this time there were no immediate prospects of a general Physicians vacancy at St George's though, through Wilfred Fox, the then senior dermatologist, the possibility of studying skin diseases was suggested. Hugh Gordon took to the idea and once he had married Jean Robertson at St George's Hanover Square, he set off for Paris where he studied at the St Louis Hospital for a year. Paris was followed by a shorter spell in Vienna, the other great centre for dermatology and there he was introduced to the technique of radio- or Rontgen-therapy; this was being vigorously developed on the Continent and later proved to be of great use in his practice—it was surely a factor for his particular interest in the work at the Cancer Hospital (now the Royal Marsden) where he became an acknowledged expert. On his return he quickly obtained three hospital appointments: St John's Hospital, Lewisham, the Shadwell Children's Hospital and the East Ham Memorial Hospital; these would have been for one clinic a week, even a fortnight.

In 1933 he was appointed Assistant Physician to the Skin Department of St George's. There were then two outpatient clinics a week and four beds available for in-patients—hardly one of the busiest of the London clinics. Venereology had not, at this time, been separated from dermatology and it was Hugh Gordon's lot to take some of the 'Special' evening Clinics. With this change in his status he relinquished his first three consultancies and was quickly appointed to two more prestigious hospitals, the Cancer in Fulham Road and the West London, which was the second and smaller teaching Hospital for women. Thus, his 'parish' was conveniently compacted in West London and his practice understandably thrived. Despite a large private practice he nevertheless found time to be Assistant Editor to the British Journal of Dermatology.

The 1939 war brought total upheaval of medical arrangements in London. Ten administrative Sectors were designed, fanning out from a teaching Hospital at the central point, to peripheral hospitals at the base. Children and families were evacuated, bombing and the blitz were to be anticipated and the St George's patients went to the West Middlesex and other hospitals in the West. Hyde Park Corner was empty of patients and was to serve as a casualty reception station.

Teaching of students had to continue in a largely empty hospital and it was at this time that Gordon showed his organizational abilities. He assumed the general physician's role, he supervised undergraduate instruction and he became Dean of the Medical School. It was largely due to him that the School survived in those difficult times for its total demise had been suggested more than once. In addition he was the dermatologist for that particular Sector of the E.M.S. visiting a number of hospitals on a regular basis. At the South West London Fever Hospital in Twickenham there was a military Skin Unit in his charge. This was the time of the dyes so the young patients were painted in multicoloured hues with yellow acriflavine, crystal violet, brilliant green and scarlet red much in evidence. The ward was known as the Harlequin.

By the time peace was established Gordon had kept the Medical School alive. He was to continue as the Vice-Dean for a while, furthermore he was responsible for introducing the first lady students from Oxbridge to what had, hitherto, been an all male preserve. An outstanding cohort they proved to be.

The National Health Service was to come about in 1948 and the future of St George's was once again under scrutiny. Hugh Gordon continued to be a powerful and effective witness in its defence. It was natural that he was to become Chairman of the Medical Committee and for many years a member of the Board of Governors of the Hospital. Indeed many of the other Board members, men of affairs, were his personal friends. From the earliest days he had been a keen advocate for the idea of St George's moving out of Central London, once the possibility of rebuilding at Hyde Park Corner had been abandoned.

In the 1950s his practice was thriving, indeed he had two consulting rooms—one in Harley Street and one in Belgravia, something which was then quite unusual. He employed both a nurse/radiotherapist as well as a secretary. He was now a Senior figure in the dermatological establishment so he was naturally to become President of the Section at the Royal Society of Medicine and later, the President of the British Association of Dermatologists.

Throughout, he maintained a great interest in malignant diseases of the skin, in all their aspects. He was not a prolific writer though contributed the chapter on skin tumours in the 'Modern Practice of Dermatology' edited by Mitchell-Heggs (1950). He frequently presented cases at Clinical meetings and for a time became intrigued with the notion that some skin diseases could be modified by hypnosis.

Later in the 1960s he was to move to Yately to his father's old home. It was a large property which enabled him once more to indulge his horticultural interests. He finally sold the estate and returned back to his original Scottish roots, happily living in the High Street of Kirkcudbright. Jean, his wife survives him along with his two married children Grahame and Huguette. Hugh Gordon was to the end a remarkable man, tall, agile, uncommonly handsome, courteous and friendly. This gentleman doctor of yesterday was a splendid servant of his old teaching Hospital and Medical School.

S.G.

Pediatric Dermatology

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Discoid lupus erythematosus associated with the anticardiolipin syndrome

SIR, Discoid lupus erythematosus (DLE) and the anticardiolipin syndrome are well known associations of systemic lupus erythematosus (SLE), but have not previously been reported together in the absence of SLE. We wish to report a case of DLE associated with the anticardiolipin syndrome.

Mr RT developed clinically characteristic DLE of the face in 1972 at the age of 29. The diagnosis was confirmed by conventional histology and immunofluorescence.

In May 1983 he was admitted for investigation of pleuritic chest pain, breathlessness and haemoptysis. He gave a history of swelling and pain in the left calf and thigh and two previous episodes of 'pleurisy'. Thrombosis of the left femoral vein was demonstrated by venography. He was treated with heparin and warfarin, and made a full recovery. Warfarin was discontinued in January 1984. In October 1985 he was readmitted with a recurrence of venous thrombosis confirmed by venography. On neither occasion was there any apparent predisposing factor to venous thrombosis. He has subsequently remained on warfarin.

Investigations during his second admission gave the following abnormal results: clotting studies consistently demonstrated lupus anticoagulant activity; anticardiolipin antibody was positive at between 4 and 5 standard deviations above the normal range for IgG and between 6 and 7 for IgM; cryoglobulin was present at the moderately raised level of 2·9 g/l containing polyclonal IgG and IgM. Normal results included negative anti-nuclear factor, anti-Ro and La antibodies; immunoglobulin levels, C3, C4; antithrombin III levels by functional and immunological assays; protein C, β-thromboglobulin, and platelet factor 4.

This patient has suffered recurrent venous thrombosis associated with lupus anticoagulant activity and anticardiolipin antibody. He, therefore, has the anticardiolipin syndrome.[1] He also has long-standing DLE but does not have sufficient clinical, haematological and serological features for the diagnosis of SLE,[2] despite the DLE having been present for 15 years. There is no previous report of such a case, although Mayou *et al.* reported the presence of anticardiolipin antibody, in the absence of clinical features of the syndrome, in eight of 52 patients with DLE.[3]

The question arises as to the nature of the inter-relationship between the anticardiolipin syndrome, DLE and SLE. The anticardiolipin syndrome and DLE may occur independently as distinct disease entities.[1,4] In addition, the features of the anticardiolipin syndrome and DLE are well known associations of SLE, but this is the first report of the anticardiolipin syndrome and DLE alone. In view of the high incidence of raised anticardiolipin antibody titres reported in DLE,[3] we consider it unlikely that DLE and the anticardiolipin syndrome are associated simply by chance in this patient. The evidence suggests a genuine association between these two diseases.

The presence of laboratory abnormalities is generally considered not to affect the prognosis in patients with DLE,[4] but the detection of anticardiolipin antibody may prove to be an exception. In addition it would seem necessary that patients with DLE, who develop venous thrombosis, should be investigated for the presence of the anticardiolipin syndrome.

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REFERENCES

1 Sontheimer RD. The anticardiolipin syndrome. *Arch Dermatol* 1987; **123:** 590–5.
2 Tan EM, Cohen AS, Fries JF *et al.* The 1982 revised criteria for the classification of systemic lupus erythematosus *Arthritis Rheum* 1982; **25:** 1271–7.
3 Mayou SC, Wojnarovska ET, Lovell CR *et al.* Anticardiolipin and antinuclear antibodies in discoid lupus erythematosus: their clinical significance. *Br J Dermatol* 1987; **116:** 445.
4 Rowell NR. Lupus Erythematosus. In: *Textbook of Dermatology.* (Rook AJ. Wilkinson DS, Ebling FJ *et al.*, eds), 4th edn., Vol. 2. Oxford: Blackwell Scientific Publications, 1986; 1282–334.

Rare-earth doped eyewear provide for safer laser surgery

SIR, The copper vapour laser is a powerful source of green (511 nm) and yellow (578 nm) light. It is agreed on theoretical grounds that yellow light (570–585 nm) is optimal for the treatment of port wine stains.[1] Some clinical and histological evidence supports this.[2–4] With our laser, a Quentron™ QM90-51C copper vapour laser, the 511 nm line is all but completely filtered from the beam before the 578 nm emission is focused into an optical fibre. Thus, only protection from the 578 nm light is required. Previously we have used red transmission filters in all our protective eyewear (such as Schott™ RG 610[5]). Although filtering out the required wavelengths these glasses have a number of disadvantages. First the transmission of visible light through such filters is only about 5–10% of the total visual response of the eye.[6] Second, this restricted vision means there is a strong temptation to remove the glasses in the theatre when there may appear to be a minimal eyesight risk. Third, the port wine stain cannot be distinguished from normal skin. Four, the lesion's immediate response to the light cannot be observed. This is particularly crucial when the desired endpoint to treatment depends on the observation of the skin's response.

Complete protection[7] from direct visual exposure to 6 W of 578 nm light is afforded by eyewear attenuating the light to a corneal irradiance of $50\ \mathrm{mW/cm^2}$. Using a standard 7 mm pupil diameter, the limiting exposure level requires an attenuation of $3{\cdot}2 \times 10^{-7}$ of the incident light. Given the parameters of beam size and the light delivery system adequate protection is achieved by an attenuation of 10^{-6}. We have found we are able to provide adequate protection and good overall vision with 1·5 mm thickness Schott BG 36 filter glass. This rare-earth doped glass has a narrow absorption band between 560 and 600 nm. However, overall there is a 30–35% effective visual transmission and essential normal colour vision is preserved. The slight leakage of green light from the fibre optic provides no danger to the eye but does impair the surgeon's view of the spot being treated. This problem can be easily rectified with the addition of 1 mm thickness Schott OG 550 filter glass. OG 550 is essentially totally opaque to visible wavelengths below 530 nm but is at least 80% transparent above 550 nm.

These filters have greatly enhanced the safety standards within the operating theatre. Furthermore, they have given the surgeon the ability to interactively assess the treatment progress and to apply the light more accurately.

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REFERENCES

1 van Gemert MJC, Welch AJ, Amin AP. Is there an optimal treatment for port wine stains? *Lasers Surg Med* 1986 **6(1)**, 76–83.
2 Morelli JG, Tan OT, Garden J, Margolis R *et al.* Tunable dye laser (577 nm) treatment of port wine stain. *Lasers Surg Med* 1986 **5(2)**: 94–9.
3 Goldman L, Taylor A, Putman T. New developments with the heavy metal vapour lasers for dermatologists. *J Dermatol Surg Oncol* 1987 **13(2)**: 163–5.
4 Cotterill JA. Preliminary results following treatment of vascular lesions of the skin using a continuous wave tunable dye laser which emits at 577 nm. *Clin Exp Dermatol* 1986, **11**: 628–35.
5 Schott. Optical glass filters, Schott Claswerke, Mainze, Federal Republic of Germany 1984.
6 Wysecki G, Stiles WS. *Colour Science: Concepts and methods, qualitative data and formulae.* (2nd ed). New York: Wiley, 1982.
7 AS 2211 Australian standard AS 2211 Laser Safety, Standards Association of Australia, North Sydney, NSW, Australia 1981.

Successful treatment of genital and oral ulceration in Behcet's disease with topical 5-aminosalicylic acid (5-ASA)

SIR, We report a case of Behcet's disease that had topical 5-aminosalicylic acid (5-ASA) as the sole therapy.

The patient, a 24-year-old woman, had suffered from aphthous stomatitis, genital ulcers, cutaneous and subcutaneous erythematous nodules and small blisters since 1983. The diagnosis of Behcet's disease was based on Mason and Barnes criteria.[1] In 1987, the genital and oral lesions became progressively worse and she developed a deep ulcer on the inside of the lower lip extending for about 2 cm with loss of substance and destruction of the frenulum. Another ulcer of similar size developed in the right inferior retromolar area and both lesions were intensely painful and made eating difficult. The patient lost 4 kg in weight over a period of 2 months. An irregular extensive ulcer was also present in the right labium minus of the vulva with an underlying abscess and loss of tissue, with half of the labium minus disappearing. This lesion was also extremely painful. A biopsy of the labial lesion was taken and showed the histology of acute and chronic inflammation with a vasculitis and occasional multinucleate giant cells. She had no other symptoms, but there were a number of erythematous nodules, mainly localized on the buttocks and forearms that were intensely itchy. Routine blood investigations and immunological tests (C_3, C_4, anti-nuclear, anti-mitochondrial, anti-smooth-muscle antibodies) were all negative.

Behcet's disease is an inflammatory vasculitis characterized by recurrent multisystem lesions and of unknown aetiology. Various mechanisms have been suggested for the aetiology both for Behcet's disease and ulcerative colitis[2] and in the latter disorder, topical treatment with 5-ASA is often effective.[3] We therefore tried topical 5-ASA on our patient with Behcet's disease.

From October, 1987, we treated the oral and vaginal ulcers with topical applications of 5-ASA in water. A gauze poultice was applied twice daily to the ulcers, the gauze being impregnated with a suspension of 5-ASA using a total of 1·5 g daily of the granules. There was a progressive healing of the ulcers and after 10 days she found eating much easier and started to gain weight. She was able to walk without discomfort and there was complete healing of the lesions within 50 days. Our patient still continues on treatment with topical 5-ASA and the ulcers have not recurred. We suggest that topical 5-ASA may have an important role in the treatment of mucosal lesions of Behcet's disease.

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REFERENCES

1 Mason RM, Barnes CG. Behcet's syndrome with arthritis. *Ann Rheum Dis* 1969; **28:** 95–103.

2 Kirsner JB, Shorter RG. Recent developments in nonspecific inflammatory bowel disease. *N Engl J Med* 1982; **306:** 775–85, 837–48

3 Willoughby CP, Piris J, Truelove SC. The effect of topical N-acetyl-5-aminosalicylic-acid in ulcerative colitis *Scand Gastroenterol* 1980; **15**; 715–9.

4 Basilisco G, Ranzi T, Campanini M *et al.* 5-aminosalicylic acid or sulfasalazine retention enemas in distal ulcerative colitis. A randomized therapeutic trial. *Current Therapeutic Research* 1987; **42:** 910–915.

Cataracts associated with long-term topical steroids

SIR, Black and co-workers[1] were the first to describe the development of posterior subcapsular cataracts in patients affected by rheumatoid arthritis treated with corticosteroids. Since then, steroid-induced cataracts in patients receiving systemic therapy have been well documented.

Nielsen and Sorensen[2] reported a case of glaucoma induced by application of corticosteroids to the periorbital region and more recently, a close relationship was found[3] between glaucoma and long-term application of corticosteroids to the eyelids. In these cases, the drugs were applied around the eye region and, thus, it is difficult to establish whether they acted directly on the eye, or whether they acted through a systemic route.

We report three cases of cataracts induced by long-term glucocorticoid cutaneous application. As far as we are aware these are the first cases presenting clear evidence that application of glucocorticoids on the skin can cause cataracts.

Case MA. A 56-year-old man with psoriasis applied a paste containing fluorometholone (0·25%) to his skin for 2 years at a dose of about 1 g/day. At slit lamp observation before glucocorticoid therapy both lenses were normal and clear. However, after 2 years the patient presented with posterior subcapsular cataracts affecting both eyes.

Case AG and case AA were two brothers aged 45 and 47 years with X-linked ichthyosis. They were periodically observed with a slit lamp for the presence of corneal opacities.[4] Both had applied a paste containing fluorometholone (0·25%) to their skin for 2 years using about 1 g/day. Before this treatment the lenses were normal and clear, but after 2 years both had posterior subcapsular cataracts in their eyes.

The cataracts in our patients were not due to the dermatological disease and lens opacification has not been reported as an ophthalmological finding in psoriasis and X-linked ichthyosis.[4] The location of the opacities suggests a relationship between topical steroid therapy and the cataracts. Though glaucoma may be associated with systemic steroid therapy, the topical application of corticosteroids is thought not to have adverse effects on the eyes.[5] In our patients the long-term cutaneous application of corticosteroids however, appeared to induce the posterior subcapsular cataracts. In our cases it seems likely that the drugs acted systemically.

The pathogenesis of steroid-induced cataracts is obscure but recently it has been shown that the first event, as regards the biochemical changes occurring in steroid-induced cataracts, is a fall in the levels of protein and non-protein sulphhydryl groups of the lens.[6]

The cases described may be considered extreme, given the prolonged period of therapy and the amounts of topical steroids used, but they illustrate the importance of such patients having periodical ophthalmological examinations.

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REFERENCES

1 Black RL, Oglesby RB, von Sallman L., Bunim JJ. Posterior subcapsular cataract induced by cortiscosteroids in patients with rheumatoid arthritis. *J Am Med Ass* 1960, **174:** 166–71.
2 Nielsen NV, Sorensen PN. Glaucoma induced by application of corticosteroids to the periorbital region. *Arch Dermatol* 1978; **114:** 953–7.
3 Vie R. Glaucoma and amaurosis associated with long-term application of topical corticosteroids to the eyelids. *Acta Dermatol* 1980; **60:** 541–4.
4 Fitzpatrick TB, Eisen AZ, Wolff K, Freedberg IM, Austen KF (eds). *Dermatology in General Medicine*, 3rd edn. New York: McGraw Hill 1987; pp. 472–507.
5 McDonnel PJ, Kerruir MG. Glaucoma associated with systemic corticosteroid therapy. *Lancet* 1985; **ii:** 386–7.
6 Costagliola C., Iuliano G, Menzione M *et al.* Effect of topical glucocorticoid administration on the protein- and non-protein-sulfhydryl groups of the rabbit lens. *Ophthalmic Res.* 1987; **6:** 351–6.

Seventy years on: 3, St Andrew's Place

SIR, A visit to the spanking and spacious new offices at 3, St Andrews Place prompts this ageing ex-Secretary to ponder on the Association's fortunes and to marvel at the transformation of such a small, modest gathering to this thriving, efficient and affluent affair. There is no doubt that the modern B.A.D. with its wide influence, its philanthropic support of research and travel and its close involvement with the current medico-politicial 'hot potatoes' is there for all to see. Our meetings attract visitors from far and wide, and without doubt, the standard of presentation at our meetings—the clarity and detail, the careful rehearsal and impeccable timing are recognized as the hallmark of our members. The *British Journal of Dermatology* is the standard that flies proudly over our activities.

What are the landmarks which stand out in this evolutionary journey? Sir Archibald Gray first suggested the formation of this Association in 1919, just after the First World War had ended and the first meeting was in 1921. Gray was a busy and efficient clinician and wrote prolifically, but administration seemed to have been his forte. He was Home Treasurer for 20 years but as well, had been Editor from 1916 to 1929 and President in 1938. The funds of the association were skilfully managed under his guidance and, being particularly keen to keep a close relationship with general medicine, he conceived the idea that a financial gift to the R.C.P., then moving from Trafalgar Square to Regent's Park (and looking for funds), might earn us grateful acknowledgment and a permanent foothold in the new college. So it proved to be and the Willan Room is the result. Though it is not exclusively our preserve, we do house three portraits there (Erasmus Wilson, Sir Archibald Gray and Geoffrey Dowling), also our library and other items of interest. Outside in the corridor hangs the portrait of Robert Willan, loaned by the Governors of Sedburgh School, and a large portrait of John Ingram presented by his widow, Dame Kathleen Raven. The Willan Room had been the meeting place of our executive since the College first opened.

John Ingram followed Gray as Treasurer, indeed he held office for many years. At the end of the Second World War the Association's funds had been successfully tended, but they were still modest by current standards. They stemmed from subscriptions (the total membership at this time was no more than 60) and the sales of the B.J.D., still published by H.K.Lewis. After the war finished and the N.H.S. was introduced in 1948 all specialists increased in number, a great surge of post-war consultants were to be appointed. This was the time when Geoffrey Dowling, assisted by Hugh Wallace, was attracting numbers of ex-service trainees to St Thomas's and St John's in London and in fact both were to be Secretaries of the B.A.D. At this time of growth the Secretary's functions were almost limitless. We were moving from a select, elite association and no longer were we restricting applications for either social, academic or other reasons. Anyone totally committed to dermatology was accepted and the black balls were thrown away. Our funds were carefully guarded and investments expertly handled by each of our Treasurers be they Ingram, Oliver Scott or Harvey Baker.

But where had these increasing supplies of money come from? Two sources—the Journal and commerce. The turning point for the B.J.D. was undoubtedly moving to Blackwells as publisher. This was the master stroke conceived by a brilliant Editor, Arthur Rook, one of Dowling's outstanding pupils. Without doubt he was and is the great name, known world wide, a wonderful dermatological ambassador for this country. Now the Journal has become transformed, its management highly efficient, its circulation increased and the profit therefrom considerable.

It is not difficult to understand that in those earlier spacious days the idea of commercialism, trade exhibitions and advertising were anathema to the older generation. Finally, and against considerable opposition, it was decided to follow what had been happening elsewhere in the world and that was to accept the offer of the pharmaceutical firms to help financially. Early ones in the field were Glaxo and Stiefel, both of whom continue to provide considerable support. The meeting at Oxford when Renwick Vickers was President was to some extent assisted by outside funds for the first time. Later meetings had proper trade exhibitions and these are now totally accepted as normal. It was this particular change that has transformed our economic position. Our swelling coffers, aided by judicious investment have enabled the Association to fund many travel grants, bursaries, scholarships and the like. Our yearly accounts make impressive reading.

During this time the executive had also to keep pace and, whereas, the Honorary Secretary still does a 5 year stint he is now aided by an expert professional staff. Peggy Paxton, who had been for years at the R.S.M. was the first paid professional and now Linda Barducci (ex St John's) has two other ladies under her command. Our first official 'Office' (as opposed to the Secretary's den/study or Consulting room) was at John Street in a basement provided by Blackwells. It was obvious that this was only to be a stopgap and admirable it was, but now the present accomodation within the medical precinct of the College is ideal and surely would cause Archibald Gray, John Ingram, Geoffrey Dowling and Hugh Wallace great delight. Go and have a look at them, make yourself known and admire the fruits that stem from the foresight and labours of a few far-sighted men.

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London W1N 2DE, U.K.

S.Gold

British Journal of Dermatology (1989) **120,** 475–477.

Book Reviews

Hair Transplantation. Edited by W.P.UNGER AND R.E.A.NORDSTROM. 2nd edition. New York: Marcel Dekker. Pp. 768. Price $150.00.

This is the second, much expanded, edition for which Rolf Nordstrom, Department of Hand and Plastic Surgery, Umea, Sweden, has joined Walter Unger as co-editor. There have been many books on hair transplantation during the last decade but 'Unger' has been considered one of the important contributions to this highly specialized branch of surgery. A second edition is, therefore, very welcome. It will be even more well-received than the first because much of the increase in size relates to the many new techniques evolved during the 1980's in relation to reduction, flap and tissue expansion techniques—over 100 of the 768 pages are on these subjects.

The editors deserve enormous credit for producing such a detailed authoritative book—even though 47 authors have contributed there is surprisingly little repetition or omission.

It is always easy to proffer criticism of multi-author textbooks—no editor could hope to get everything right, and of uniform high quality, with 47 authors to deal with! The book would probably have been improved by a more detailed index; some colour pictures, particularly of surgical techniques; more detail on all aspects of complications, terribly important in any surgical text.

Only one part offended the reviewer; page 752 has several paragraphs describing quite specific dietary exclusions said to improve hair oiliness and suggesting high dose vitamin B to combat the effects of 'stress' on hair. Such 'pseudo-therapeutic' anecdote is surely best reserved for less academic publications than this important book.

R. DAWBER

Difficult Diagnoses in Dermatology. Edited by M.LEBWOHL. (1988) New York: Churchill Livingstone. Pp. 450. Price £50.00.

We all develop strategies for trying to solve problems of differential diagnosis. This multi-author book has 23 chapters covering a range of subjects that may at times be difficult.

The most useful sections are those dealing with topics which are thinly spread in the major textbooks, such as 'fever and rash' and the erythrodermas, pustular diseases and bullous dermatoses in childhood. Also of interest are the accounts on hirsutism and chronic urticaria, where there are clear expositions of the when and why of tests in relation to clinical findings.

The book is less successful with topics which lend themselves to more straightforward textbook description, such as the alopecias, annular erythemas and scleroderma.

The book would be improved if there was more on the practicalities of making diagnoses and less on pathophysiology and treatment—aspects more thoroughly dealt with in other texts.

There are many useful tables for quick reference, and the text is easy to read. The book can be recommended for a departmental library but may seem rather expensive for the individual dermatologist.

C.KENNEDY

The Melanotropins: Chemistry, Physiology and Mechanisms of Action. A.N.EBERLE (1988) Basel: S. Karger. Pp. 544. Price £145.00.

There have only been a few books on the melanotrophins and considering the renewed interest in these peptides this book is most timely. It contains chapters on the chemistry of the MSH peptides, methods of assay, their physiological roles and an excellent chapter on mechanisms of action. There is an extensive and up-to-date bibliography of over 2000 references, many of which relate to very recent works. A most useful feature of the book is the provision of detailed practical information and this will make the book extremely useful as a laboratory manual.

Most dermatologists will recognize the melanotrophins as pigmentary hormones but it is becoming increasingly clear that these peptides have many different effects, including actions on the nervous and immune systems. This book covers all these topics and should provide a good introduction to the subject although I suspect it will have greater appeal to established MSH researchers. Whether they will be able to afford the book is another matter!

A.J.THODY

Surgical Application of Lasers. (2nd Edition) Edited by J.A.DIXON (1987) Chicago: Year Book Medical Publishers. Pp. 376. Price £45.50.

In this second edition Dr Dixon and his authors have extended and updated the text to provide a very readable, comprehensive account of the currently accepted clinical uses of lasers. All specialities, except ophthalmology, are covered and there are excellent chapters from the editor on how to establish a laser unit (if only one had the chance!) and from Professor John Parrish on laser photomedicine, although I think the fundamental aspects could usefully have been discussed in more detail. Practical laser safety correctly merits its own chapter, and the chapter on photodynamic therapy, if a little uncritical, is wide ranging and well referenced. John Ratz gives us a good overview of dermatological applications and describes for the first time the RATZ (Roving Automated Treatment Scanner?) system in which a tunable laser, in combination with a sensing probe and PPA (Perfect Phototoxic Agent) treats a single patient's basal cell epithelioma, port wine stain and psoriasis automatically in a matter of minutes! Maybe this aspect of the book is a shade too fanciful but perhaps a major plus is that nowhere will you find any mention of T4/T8 ratios, 12-HETE, EGF of calmodulin! If you are thinking of starting to work with lasers, or decided that now is the time to learn a little more about them, this book is an excellent beginning.

N.P.J.WALKER

Computing for Clinicians. T.CHARD (1988). London: Elmore-Chard. Pp. 136. Price £18.00.

This book is a substantial review of the published uses of computers in medical environments. The account, which assumes no previous knowledge of computing, includes much of that basic information which is easy for the self-taught 'expert' to overlook. Professor Chard explains important concepts clearly in some detail and avoids information which the medical user will not require.

Six major types of application are covered, ranging from medical administration through diagnostic systems to computer-assisted learning. Some of these are still at an early stage in their development towards usefulness. This account is valuable because it includes reminders of many of the points which have been found in practice to cause difficulties. There is a short index and a useful list of over 150 references dated up to 1987.

Although this is not a 'cook-book', I recommend it for everybody who wishes to become involved in setting up a system which includes the use of a computer.

MARGARET CORBETT

Das AIDS Virus. KARIN MÖLLING. (1988) Weinheim: VCH. Pp. 265. Price DM 48.

The so-called AIDS epidemic has, in the last few years, resulted in an epidemic of publications on AIDS and HIV infection. Scarcely a month goes by without a large number of new volumes of varying format and quality arriving in the bookshops. This particular book is beautifully produced and covers a large amount of information, much of it very up-to-date. There are chapters on the virus itself, its infectivity, clinical manifestations of HIV infection and various aspects of treatment. There are extremely useful later chapters and appendices which give details of the CDC classification of HIV-related illnesses, the Walter Reed staging system and the 1987 CDC revision of the criteria for AIDS itself. The layout of the book is excellent and the illustrations are among the best I have ever seen in a publication of this size and format. The text is also clear and I thoroughly recommend the book to all those with a basic knowledge of German. I hope that the publishers in due course consider an English translation thus making the excellent information in the book available to a wider audience.

N.P.SMITH

Kaposi's Sarcoma: A Text and Atlas. Edited by G.GOTTLIEB AND A.B.ACKERMAN. (1988) Philadelphia: Lea and Febiger. Pp. 330.

There can be scarcely any practising physician who has not become more aware of Kaposi's sarcoma since the recognition of the Acquired Immune Deficiency Syndrome in the early 1980s. In the last few years there has been a dramatic increase in interest in this remarkable neoplasm. There has even been controversy regarding whether Kaposi's sarcoma is in fact a neoplasm or an unusual form of reactive proliferation. In addition to numerous individual papers on the subject there have been several monographs recently published. This beautifully illustrated volume edited by Drs Gottlieb and Ackerman is a most useful addition to the available literature. Gottlieb and Ackerman were among the first to recognize the occurrence of Kaposi's sarcoma in young male homosexuals and their seminal article published in 1981 forms an appropriate appendix to the book. The illustrations and the text cover most aspects of clinical and histological diagnosis, epidemiology and immunopathology of the tumour and a total of 26 authors have contributed to the various sections. Juan Rosai's introduction, 'A Riddle Within a Puzzle' discusses some of the controversies surrounding Kaposi's sarcoma and there is an interesting historical and biographical account of Kaposi himself by William Ober. The substantial chapters four and five, written by the editors, covering the clinical and microscopic features of the tumour and other lesions that may simulate it are amongst the best in the book. The superb illustrations are clearly laid out and the discussion and legends are appropriate and helpful. In particular the clinical and pathological features of the early stages of the disease are well demonstrated. There are several contributions on Kaposi's sarcoma not associated with HIV infection, particularly with reference to the disease as it is seen in Africa. As is inevitable in a multi-author text book such as this, there is some overlap and unevenness between individual chapters. Although the book is entitled *A Text and Atlas,* I personally would have liked to have seen a fuller discussion of staging and therapy of AIDS. I appreciate, however, that this might not fall within the scope of an atlas. I was a little surprised to see chapters on infectious diseases in AIDS, and malignant lymphoma in AIDS, in a monograph of Kaposi's sarcoma. The major part of the chapter on malignant lymphoma consists of eight case reports and the discussion is rather superficial and unfortunately now rather out of date. Despite these comments *Kaposi's Sarcoma: A Text and Atlas* is a very attractive book and complements well the other interesting monographs on this subject such as the recent excellent book edited by Ziegler and Dorfman. It can be strongly recommended.

N.P.SMITH

Books Received

Low Level Laser Therapy: a Practical Introduction. T.OSHIRO AND R.G.CALDERHEAD (1988) Chichester: John Wiley. Pp. 143. Price £30.50.

British Journal of Dermatology (1989) **120,** 478.

News and Notices

18th World Congress of Dermatology—New York, 12–18 June 1992

The next (18th) World Congress of Dermatology will take place in New York City 12–18 June 1992. 'Dermatology—Progress and Perspectives' is the theme of the 6-day programme, focusing on the most recent advances and important issues in worldwide dermatology, and future directions in research and therapy. The Congress will provide a stimulating educational experience and a unique opportunity for dermatologists to interact with colleagues from all over the world.

John S.Strauss, M.D., has been named President of the Congress, and Stephen I.Katz, M.D., Ph.D., will serve as the Secretary General. Honorary Presidents are Rudolf L.Baer, M.D., and Clarence S.Livingood, M.D.; Alan R.Shalita, M.D. is the Deputy Secretary General.

The preliminary programme, abstract forms, and registration materials will be available in December 1990. Additional information can be obtained from the 18th Congress Secretariat, 22 Euclid Street, Woodbury, NJ 08096, U.S.A.

An International Update in Dermatology and Dermatopathology

An International Update in Dermatology and Dermatopathology, organized by the Pathological Society of Great Britain and Ireland and the British Society for Dermatopathology, will be held in Brighton, U.K. from 2 to 5 September 1989. The meeting will include sessions on the following topics: skin appendage tumours (Chairman Dr N.P.Smith), non-epithelial tumours (Chairman Dr J.T.Headington), melanocytic tumours (Chairman Professor R.M.MacKie), pathogenesis of inflammatory dermatoses (Chairman Professor N.A.Wright), cutaneous lymphoid infiltrates (Chairman Dr A.B.Ackerman), immunologically mediated skin diseases (Chairman N.Kirkham), vasculitides (Chairman Professor E.Wilson Jones), epidermolysis bullosa (Chairman Dr P.H.McKee) and acquired bullous dermatoses (Chairman Professor M.W.Greaves). There will be two slide seminars. Further details available from: Dr P.H.McKee, Department of Histopathology, St Thomas' Hospital Medical School, Lambeth Palace Road, London SE1 7EH, U.K.

Cell and Tissue Kinetics

A Journal for Cell Proliferation

The Journal of The Cell Kinetics Society,
The European Study Group for Cell Proliferation
and The International Cell Cycle Society

Edited by C.S. Potten
(European Editor) and
Z. Darzynkiewicz
(North American Editor).

Cell and Tissue Kinetics is devoted to studies of cell proliferation and differentiation in normal and abnormal states; of control systems and mechanisms operating at inter- and intracellular as well as at molecular levels; their modification by chemical and physical agents. In addition to complete papers the Journal publishes brief communications (of up to 500 words) and invited review articles.

Subscription Information

Cell and Tissue Kinetics is published bi-monthly. Subscription rates for 1989 are £97.50 (UK), £117.00 (overseas), $197.50 (USA & Canada) post free.

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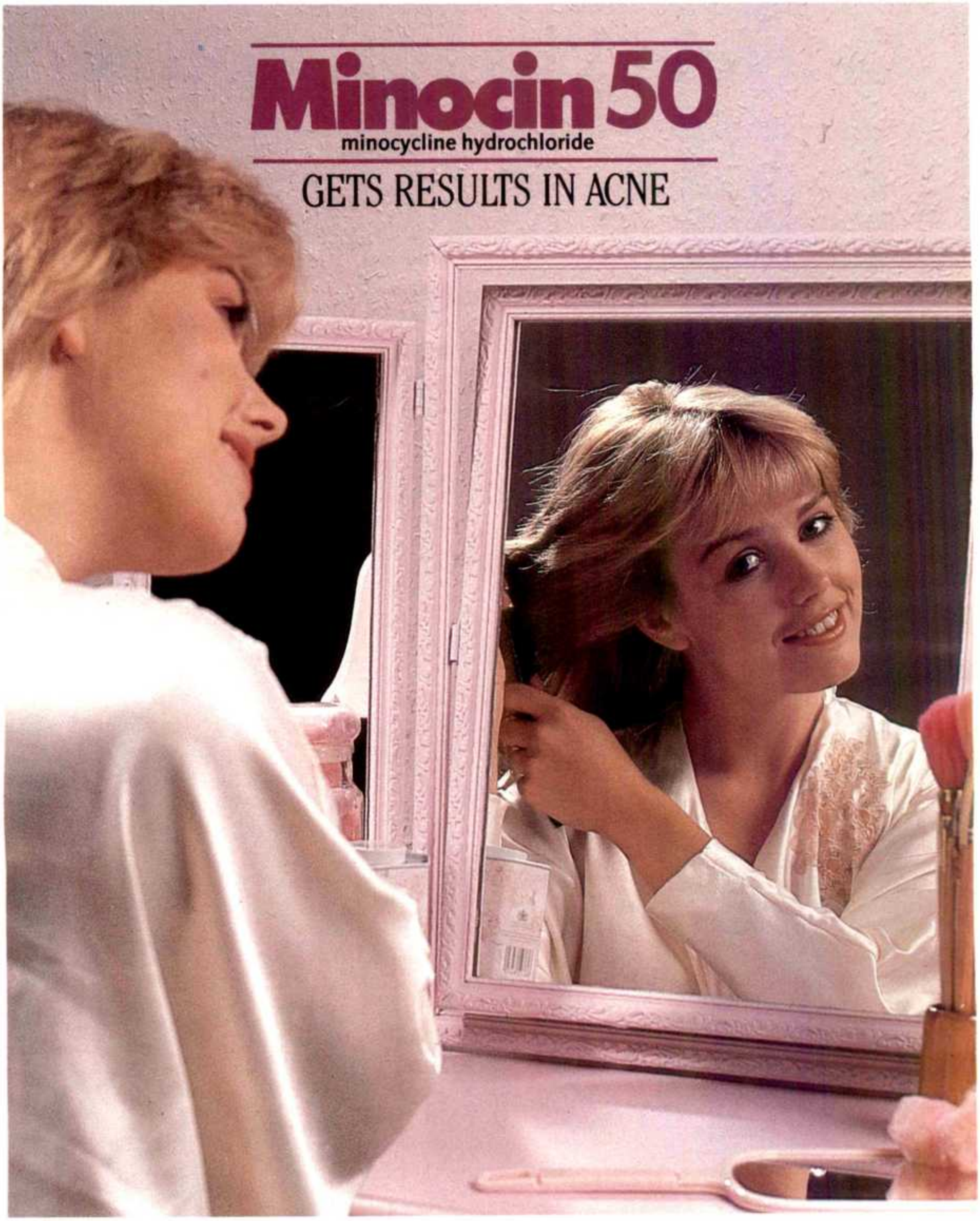

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NOTICE TO CONTRIBUTORS

The *British Journal of Dermatology* publishes original articles on all aspects of the normal biology, and of the pathology of the skin. Originally the Journal, founded in 1888, was devoted almost exclusively to the interests of the dermatologist in clinical practice. However, the rapid development, during the past twenty years, of research on the physiology and experimental pathology of the skin has been reflected in the contents of the Journal, which now provides a vehicle for the publication of both experimental and clinical research and serves equally the laboratory worker and the clinician. To bridge the gap between laboratory and clinic the Journal publishes signed editorials reviewing, primarily for the benefit of the clinician, advances in laboratory research, and also reviews of recent advances in such aspects of dermatology as contact dermatitis, therapeutics and drug reactions. Other regular features include book reviews, correspondence and society proceedings. The Journal is the official organ of the British Association of Dermatologists and also of the Netherlands Association for Dermatology and Venereology, but it attracts contributions from all countries in which sound research is carried out, and its circulation is equally international.

Papers accepted become the copyright of the Journal. This Journal is covered by *Current Contents*, *ISI/BIOMED*, *Science Citation Index* and *ASCA*.

Manuscripts. Three copies of all material for publication should be sent to the Editor, Professor S. S. Bleehen, Dermatology Department, Royal Hallamshire Hospital, Sheffield S10 2JF. Manuscripts should be typewritten on one side of the paper only, with a wide margin, be double spaced, and bear the title of the paper, name and address of each author, together with the name of the hospital, laboratory or institution where the work has been carried out. The name and full postal address of the author who will be responsible for reading the proofs should be given on the first page. The author should keep a carbon copy of his manuscript. The Editorial Board reserves the right to make literary corrections.

Abbreviations and units. Full names with uncommon abbreviations must be given with the first mention: new abbreviations should be coined only for unwieldy names and should not be used at all unless the names occur frequently. In the title abbreviations should be identified, in the introduction and discussion they should be used sparingly. Abbreviations of units should conform with those shown below.

gram(s)	g	minute(s)	min	molar	mol/l
kilogram(s)	kg	centimetre(s)	cm	millilitre(s)	ml
milligram(s) (10^{-3}g)	mg	second(s)	s	gravitational acceleration	***g***
microgram(s) (10^{-6}g)	μg	cubic millimetre(s)	mm^3	micrometre(s)	μm
nanogram(s) (10^{-9}g)	ng	millimetre(s)	mm	per cent	%
picogram(s) (10^{-12}g)	pg	millicurie(s)	mCi	isotopic mass number placed as	^{131}I
hours(s)	h	milliequivalent	mmol		

These and other abbreviations and symbols should follow the recommendations of: *Units, Symbols and Abbreviations, A Guide for Biological and Medical Editors and Authors* (1977) The Royal Society of Medicine, London.

Illustrations should be referred to in the text as 'Figs', and be given Arabic numbers, and should be marked on the back with the name(s) of the author(s) and the title of the paper. Where there is any possible doubt as to the orientation of an illustration, the top should be marked with an arrow. Each figure should bear a reference number corresponding to a similar number in the text. Photographs and photomicrographs should be *unmounted* glossy prints and should not be retouched. Colour illustrations will be accepted when found necessary by the Editor, although the author will be expected to bear the cost. Diagrams should be on separate sheets; they should be drawn with black ink on white paper or feint-ruled graph paper and should be about twice the size of the final reproduction. Lines should be of sufficient thickness to stand reduction. Each illustration should be accompanied by a legend clearly describing it; these should be grouped on a separate sheet of paper.

There should be as few tables as possible and these should include only essential data; they should be typewritten on separate sheets and should be given Arabic numbers.

References. Only papers closely related to the author's work should be referred to; exhaustive lists should be avoided. References should be given in the Vancouver style, i.e. references appear as superscript numbers in the text, e.g. '. . . our previous report[1] and that of Smith *et al.*[2] . . . ', and are brought together numerically in the reference list at the end of the article in order of their first mention in the text. References to articles and papers should mention: (1) name(s) followed by the initials of the author(s), up to four authors: if more than four authors, include first three authors followed by *et al.*; (2) title of paper; (3) title of journal abbreviated in the standard manner; (4) year of publication; (5) volume; (6) page numbers of the article. *Thus*: Cunliffe WJ, Shuster S. The rate of sebum excretion in man. *Br J Dermatol* 1969; **81:** 697–9. References to books and monographs should include: (1) author(s) or editors(s); (2) paper (if necessary) and book titles; (3) edition, volume, etc.; (4) place; (5) publisher; (6) year; (7) page(s) referred to. *Thus*: Beare JM, Wilson Jones E. Necrobiotic disorders. In: *Textbook of Dermatology* (Rook AJ, Wilkinson DS, Ebling FJ, eds), 2nd edn., Vol. 2. Oxford: Blackwell Scientific Publications, 1972; 1066.

Page proofs will be submitted without the original typescript to the author for proof-correction (see under 'manuscript') and should be returned to Blackwell Scientific Publications within three days. Major alterations from the text cannot be accepted.

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